# Investigation of the skin microcirculation in lower limb ischaemia

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*Aan mijn ouders,*
*voor Marion*

# CONTENTS

CHAPTER 1

# GENERAL INTRODUCTION

Atherosclerotic arterial obstructive disease of the lower limbs is mainly a disease of the elderly. To classify these patients clinically, the stages according to Fontaine are most commonly used (1). Asymptomatic subjects belong to stage 1. In symptomatic subjects the range of symptoms varies from moderate peripheral vascular disease, manifested as intermittent claudication (stage 2), to severe peripheral vascular disease (limb threatening ischaemia), manifested as rest pain (stage 3) or ischaemic skin lesions (ulcers and/or necrosis) with or without rest pain (stage 4). To estimate the degree of peripheral vascular disease, techniques like Doppler ultrasound, plethysmography and arteriography are routinely used (2-4). These techniques, however, only provide information about the haemodynamics and anatomy of the macrocirculation. The occurrence of ischaemic skin lesions in some of the patients with severe peripheral vascular disease is indicative of the existence of a compromised skin microcirculation besides an impaired macrocirculation.

The skin microcirculation represents the level at which the exchange between blood and tissue takes place. It is located in the dermis below the epidermis and consists of 2 parts, a nutritional part (Figure 1.1A) and a thermoregulatory part (Figure 1.1B). The combination of the nutritional skin microcirculation and the thermoregulatory skin microcirculation is referred to as total skin microcirculation.

Information about the functional state of the total skin microcirculation can be obtained indirectly by means of transcutaneous oxygen pressure ($tcpO_2$) monitoring. Direct non-invasive information about the morphology and haemodynamics of the nutritional skin microcirculation can be gathered with the use of intravital skin capillary microscopy. The relation between the degree of peripheral vascular disease, the extent of macrocirculatory impairments, and the quality of especially the nutritional skin microcirculation is incompletely understood. Ischaemic skin lesions, indicating an insufficient nutritional skin microcirculatory blood flow, do not always develop despite a severely impaired macrocirculatory blood supply. On the other hand ischaemic skin lesions do develop in patients with only limited

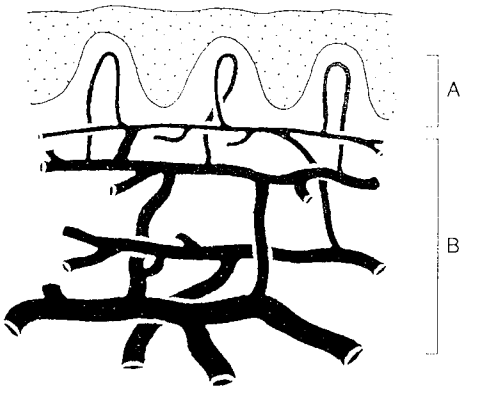


**Figure 1.1** Drawing of the skin microcirculation.
**A**: the nutritional skin microcirculation: capillaries, situated in the papillae of the dermis (papillary dermis), providing the nutrition of the skin.
**B**: the thermoregulatory skin microcirculation: a subpapillary arterial plexus, several subpapillary venous plexuses and arteriovenous anastomoses localized in the reticular dermis. The arteriovenous anastomoses, which directly connect arterioles and venules, are found in apical structures such as fingers, toes, ears, nose and palmar surfaces of hands and feet. The reticular dermis mainly deals with body temperature regulation.

macrocirculatory obstructions. These observations indicate that the cause of ischaemic skin lesions may originate from the macrocirculation and/or the skin microcirculation, emphasizing the need to investigate both levels of the circulation.

Krähenbuhl and Dubas reported that the (macrocirculatory) systolic perfusion pressure in the toe has to decrease below a value of 50 mm Hg before total skin blood flow at rest, as indicated by $tcpO_2$ monitoring, may change to subnormal levels (5). Using intravital skin capillary microscopy, Fagrell showed that skin capillaries are devoid of pronounced morphological changes despite marked reductions in systolic perfusion pressure in the toe (6,7). The impaired reactive hyperaemia following the release of a transient arterial occlusion in patients with peripheral vascular disease indicates that in these patients arterioles are dilated at rest (8-10). This autoregulatory response to local ischaemia may be regarded as an attempt to preserve an adequate nutritional skin blood supply.

The aims of the present study were to gain more insight into the relation between the degree of peripheral vascular disease and the condition of the total and nutritional skin microcirculation, and the way in which the skin microcirculation adapts to changes in macrocirculatory blood supply. The following was investigated:

1. the relation between the degree of peripheral vascular disease according to the classification of Fontaine, and objective macrocirculatory parameters, like the systolic perfusion pressure at rest and the anatomic localization of obstructions (chapter 3).

2. the relation between the degree of peripheral vascular disease according to Fontaine's classification, and objective total and nutritional skin microcirculatory parameters, like skin blood flow at rest and the degree of arteriolar dilation at rest (chapter 4).
3. the relation between total and nutritional skin microcirculatory parameters, and macrocirculatory parameters (chapter 5).

In this thesis macrocirculatory information was obtained with the use of Doppler ultrasound, plethysmography and arteriography, whereas information about the skin microcirculation was gathered by means of $tcpO_2$ monitoring and intravital skin capillary microscopy. A historical review of these macrocirculatory and skin microcirculatory investigative techniques is given in chapters 3 and 4, respectively. Information about the clinical histories and physical examinations of the subjects, who were investigated in this study, and the way, in which data are presented and statistical analysis is performed, is given in chapter 2. A general discussion concludes this thesis (chapter 6).

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# CHAPTER 2

# MATERIALS AND METHODS

In this chapter the clinical histories and physical examinations of the subjects, who participated in this study, are presented. Furthermore, the way of data presentation and statistical analysis is described. For detailed information about macrocirculatory and skin microcirculatory investigative methods, the reader is referred to chapters 3 and 4, respectively.

## 2.1 CLINICAL HISTORY AND PHYSICAL EXAMINATION

Prior to the macrocirculatory and microcirculatory investigations, data concerning the clinical backgrounds of all participating subjects were obtained by means of a questionnaire. Detailed information was collected about the nature and duration of the peripheral vascular complaints, smoking habits, and the presence of diabetes, hypertension and other current or previous diseases. An inventory was taken of all prescribed medications.

All subjects underwent a physical examination. The localization of ischaemic skin lesions (ulcers and/or necrosis) on the leg of interest distal to the knee-joint was documented. The total surface area of all ischaemic skin ulcers was determined and expressed in square centimetres ($cm^2$). At the end of the examination colour photographs of the ischaemic skin lesions were taken.

## 2.2 PATIENTS

Seventy-seven patients with moderate or severe lower limb ischaemia, referred to the hospital between May 1987 and May 1988 for evaluation of their peripheral vascular state, were investigated in this study after giving their informed consent. The median age of this group was 68 years (range: 39-90 years). It consisted of 54 men with a median age of 66 years (range: 39-90 years) and 23 women with a

median age of 72 years (range: 54-90 years). All patients were classified according to the stages of Fontaine (F).

The F2-group (stage 2) consisted of 39 patients, who suffered from intermittent claudication (moderate peripheral vascular disease). Patients of this F2-group had a median age of 64 years (range: 44-78 years).

The F3/4-group (stages 3 and 4) consisted of 38 patients. They suffered from severe peripheral vascular disease, which manifested itself in rest pain as such or in ischaemic skin lesions (ulcers and/or necrosis) with or without rest pain. The median age of these patients was 74 years (range: 39-90 years). The patients belonging to the stages 3 and 4 were combined, because they all suffered from a severely disturbed peripheral arterial blood supply at rest in contrast to the patients belonging to stage 2.

Both groups of patients are described in detail below.

### 2.2.1 F2-group (stage 2 according to Fontaine)

The 39 patients consisted of 35 men with a median age of 64 years (range: 44-78 years) and 4 women also with a median age of 64 years (range: 57-65 years). Since in this study only the most affected leg was included, the right leg was investigated in 25 patients and the left leg in 14 patients. Twenty-four patients smoked regularly. Five patients had maturity-onset diabetes mellitus. In 1 patient the diabetes was controlled by an appropriate diet, whereas the other 4 were treated with oral drugs. Eight patients suffered from hypertension. Three of them were treated with beta-blocking agents. One patient was given a combination of a beta-blocker, a vasodilator and a diuretic. A vasodilating drug combined with a diuretic was prescribed to 2 patients. In the 2 remaining patients the hypertension was controlled by a diuretic drug only. Six patients had a myocardial infarction in their clinical history. One patient had undergone coronary bypass surgery, while another patient had been subjected to percutaneous transluminal coronary angioplasty (PTCA). In 1 patient beta-blockade was used to treat angina pectoris. Previous haemodynamically successful central reconstructive vascular surgery of the leg of interest had been performed in 4 patients at least 14 months before entering the study.

### 2.2.2 F3/4-group (stages 3 and 4 according to Fontaine)

This group of 38 patients consisted of 19 men with a median age of 74 years (range: 39-90 years) and 19 women also with a median age of 74 years (range: 54-90 years). In 25 patients the right leg and in 13 patients the left leg was examined. Thirteen patients were regular smokers. Twenty-six patients had maturity-onset diabetes. In 1 patient an appropriate diet was sufficient to control the diabetes. Eighteen diabetics were treated with oral drugs, while 4 diabetics had to

be treated with insulin. In 3 patients the diabetes was managed with a combination of oral drugs and insulin. Thirteen patients had hypertension. Five of these patients were treated with a diuretic, whereas a vasodilating agent was taken by 2 patients. A combination of a vasodilator and a diuretic was prescribed to 2 patients, while 2 other patients were treated with a beta-blocker and a diuretic. A vasodilator combined with a diuretic and a beta-blocker was taken by 2 patients. Thirteen patients had a myocardial infarction in their clinical history. Three patients underwent coronary bypass surgery. In 3 patients a beta-blocking agent was prescribed to prevent angina pectoris. Haemodynamically successful reconstructive vascular surgery of the leg of interest was performed in 10 patients at least 8 months before participating in this study. A peripheral reconstructive vascular procedure was performed in 2 patients. In the remaining 4 patients reconstructive vascular procedures consisted of central reconstructive vascular procedures or a combination of central and peripheral reconstructive vascular surgery. Five patients had an amputation of one or more toes of the leg of interest. A beta-blocking agent to prevent glaucoma was prescribed in 1 patient. Thirty-three patients complained of rest pain with a median duration of 55 days. Thirty-three patients exhibited ischaemic skin lesions (ulcers and/or necrosis) on the leg of interest distal to the knee-joint. Ischaemic skin ulcers were mostly seen on toes and heels. The median total surface area of these ulcers was 2.44 $cm^2$ (range: 0.04-50.34 $cm^2$). A combination of rest pain and ischaemic skin lesion(s) existed in 28 patients. To compare the nutritional skin microcirculation in the intact skin and in rims of ischaemic skin lesions, the F3/4-group was subdivided into a F3/4-INTACT-group and a F3/4-LESION-group as far as intravital skin capillary microscopic investigations were concerned. The F3/4-INTACT- group consisted of patients (n = 27), of whom an intact part of the skin was investigated, whereas the F3/4-LESION-group consisted of patients (n = 11), on whom intravital skin capillary microscopy was performed in the rim of an ischaemic skin lesion.

## 2.3 CONTROL SUBJECTS

The control-group, consisting of 10 healthy subjects, was derived from the local community. They all gave their informed consent. Their median age was 68 years (range: 61-77 years). None of these subjects had cardiovascular complaints before or when entering the study. Six men, median age 68 years (range: 61-73 years), and 4 women, median age 68 years (range: 64-77 years) were included in this group. None of them had diabetes or hypertension. In 8 subjects the right leg and in 2 subjects the left leg was examined. Three subjects smoked regularly. None of the control subjects was taking medication.

## 2.4 DATA PRESENTATION AND STATISTICAL ANALYSIS

In this thesis the results are presented as median values. The Mann-Whitney U test was used for comparison between groups. Differences were considered to be statistically significant at $p<0.05$. Box plots and scatter plots are used as displays (1,2). As shown in Figure 2.1, a box plot visualizes the following features of a set of data:

- the median value, represented by the line dividing the box.
- the first and third quartiles, corresponding to the two values below which 25 and 75% of all values fall, respectively. The first and third quartiles are indicated by the lower and upper edges of the box, respectively.
- the 10th and 90th percentiles, which correspond to the two values below which 10 and 90% of all values fall, respectively. The 10th and 90th percentiles are represented by the lower and upper edges of the whiskers, respectively.
- stray value(s), straying out beyond the 10th and 90th percentiles. Stray values are indicated by open circles.

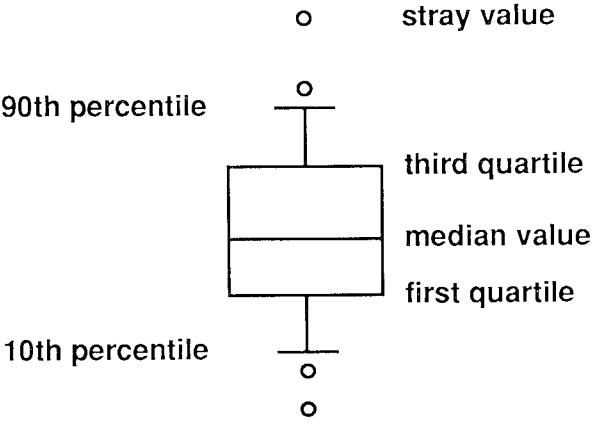


**Figure 2.1** Example of a box plot.

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# CHAPTER 3

# MACROCIRCULATION

This chapter deals with the anatomic and haemodynamic investigation of the lower limb macrocirculation by means of arteriography, and ankle and toe systolic blood pressure measurements.

## 3.1 INVESTIGATIVE TECHNIQUES

### 3.1.1 Arteriography

In January 1896, the month after Röntgen announced his discovery of x-rays, Haschek and Lindenthal introduced contrast arteriography by visualizing arteries in an amputated hand after the injection of a viscous radiopaque solution into the brachial artery (1). Brooks was the first to visualize the lower limb arteries by means of an intra-arterial injection of sodium iodide into the superficial femoral artery in 1924 (2). Visualization of the abdominal aorta and its branches was made possible by Dos Santos, Lamas and Pereira-Caldas, who brought the translumbar needle puncture and the translumbar injection of contrast medium into practice (3). The information obtained with contrast arteriography, however, was limited to the region supplied by the punctured artery. Fariñas was the first to report the retrograde passage of a catheter from the femoral artery into the aorta for aortography (4). Seldinger described a modified percutaneous transfemoral catheterization method, which has become the procedure of choice to visualize the arterial system (5). All major arteries are selectively accessible through a single puncture in the femoral or brachial artery by means of this technique. At present, percutaneous transfemoral arteriography according to Seldinger is usually applied to visualize the aorto-iliac and/or lower limb arteries. In addition, intra-arterial and intravenous digital subtraction arteriography (DSA), using photographic image subtraction to separate contrast material densities from those produced by anatomical structures, supplement or are already replacing conventional techniques.

## Methods

To visualize the aorto-iliac root, the superficial and deep femoral artery, the popliteal artery and the arteries below the knee, translumbar arteriography, percutaneous transfemoral arteriography according to Seldinger, direct puncture of the femoral artery, intra-arterial DSA, intravenous DSA or a combination of these techniques was performed on the patients in the supine position. For an extensive description of the arteriographic methods applied, one is referred to textbooks dealing with arteriography (6,7).

To classify the degree of macrocirculatory obstruction the arteriograms (single-plane projections) were reviewed by an experienced vascular surgeon, who was unaware of the outcome of the macrocirculatory and microcirculatory investigations. The leg arteries were subdivided into arteries above and below the knee. The arteries above the knee, which were reviewed separately but classified as a whole, consisted of the aorto-iliac root, and the superficial femoral and popliteal artery. They were classified as patent, if the maximum lumen reduction was 50% or less, or as obstructed, if somewhere the lumen was narrowed by more than 50% (8). The arteries below the knee, which comprised the distal popliteal artery and the anterior, posterior and peroneal artery, were classified according to Morton (9). Each of these four arteries was evaluated separately. A complete obstruction yielded a score of 0. If the lumen was narrowed by more than 75%, 50% or 25% a score was given of 1, 2 or 3, respectively. A maximum score of 4 was given if no obstruction was seen or if the lumen was narrowed by 25% or less. Then the sum of these scores was subdivided into 3 different stages of outflow obstruction. Total scores varying between 12-16, 7-11 and 0-6 corresponded with Morton-stages 1, 2 and 3 of outflow obstruction, respectively. Combining the classifications of the arteries above and below the knee provided a general arteriographic classification, which comprised 6 ARTERIOGRAPHY-GROUPS representing progressive degrees of macrocirculatory obstruction (Table 3.1).

**Table 3.1** Arteriographic classification (6 ARTERIOGRAPHY-GROUPS), representing progressive degrees of macrocirculatory obstruction. This arteriographic classification resulted from combining classifications of arteries above and below the knee.

| ARTERIOGRAPHY-GROUP | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Classification of arteries above the knee | pat | obs | pat | obs | pat | obs |
| Morton-stage of outflow obstruction | 1 | 1 | 2 | 2 | 3 | 3 |

Pat = patent, obs = obstructed; Morton-stage 1 of outflow obstruction = total score of the arteries below the knee ranges between 12 and 16, Morton-stage 2 of outflow obstruction = total score of the arteries below the knee ranges between 7 and 11, Morton-stage 3 of outflow obstruction = total score of the arteries below the knee ranges between 0 and 6.

### 3.1.2 Ankle systolic blood pressure (ASBP) and ankle-to-brachial systolic blood pressure index (ABI) measurements

Since Korotkoff established the sphygmomanometric technique by introducing an auscultatory method in 1905, blood pressure measurements have routinely been performed in clinical practice (10). For assessment of the influence of arterial obstructions on the perfusion pressure in the lower limb, plethysmographic techniques have been applied to measure the systolic blood pressure at the level of the ankle (11,12). The continuous wave Doppler ultrasound technique to measure blood flow velocity in a non-invasive way was introduced by Satomura and Kaneko (8), and Franklin, Schlegel and Rushmer (13). Clinical experience with this technique indicated, that normal arterial flow produced characteristic sounds and waveforms, which were altered in a recognizable manner by arterial narrowing or occlusion (14,15). Yao reported that the ankle-to-brachial systolic blood pressure index (ABI), measured at rest with the use of Doppler ultrasound, separated healthy subjects from patients with moderate or severe peripheral vascular disease (16). Since the ankle-to-brachial systolic blood pressure index does not indicate the relative significance of lesions at various anatomic levels, segmental lower limb pressure measurements may be carried out to resolve this shortcoming (17).

#### Methods

When an ultrasonic beam is directed at a blood vessel, emitted ultrasound is backscattered from moving (red) blood cells and its frequency is shifted proportional to the velocity of these (red) blood cells. The following formula shows the quantitative relation between the Doppler frequency shift ($\Delta f$) on the one hand and the frequency of emitted ultrasound ($f_c$), the velocity of (red) blood cells (v), and the angle between the ultrasonic beam and the direction of blood flow ($\theta$) on the other:

$$\Delta f = \frac{2 \cdot f_e \cdot V \cdot \cos\theta}{C},$$

where c is the velocity of sound in the tissues (1540 m/s for soft tissue). In this study a bi-directional continuous wave Doppler flowmeter with a zero-crossings circuitry to assess the frequency shift was used (Vasculab bi-directional Doppler, model D10, Medasonics). This Doppler flowmeter was connected to a probe, which contained a transducer with two piezo-electrical crystals: one for the continuous emission of ultrasound with a frequency of 8 MHz and one for

subsequent reception. After amplification, the detected frequency differences were presented as audiosignals.

### 3.1.3 Toe systolic blood pressure (TSBP) and toe-to-brachial systolic blood pressure index (TBI) measurements

As early as 1934, Formijne reported toe systolic blood pressure measurements in patients with peripheral vascular disease (18). A decrease in toe systolic blood pressure in patients suffering from severe peripheral vascular disease was shown by Conrad and Green (19). Carter showed that the toe systolic blood pressure correlated better with severe ischaemia than the ankle systolic blood pressure (20). This finding was confirmed by other investigators (21,22).

#### Methods

Toe systolic blood pressure measurements were carried out using a photoplethysmograph (Hewlett-Packard, Type 7830A). This device contains a photoelectric transducer, consisting of a light-emitting diode, operating in the near infrared portion of the spectrum, and a photosensor. The amount of reflected light, detected by this photosensor, varies inversely with the absorption of light by hemoglobin, which in turn is proportional to the blood volume present in the part of the skin under investigation. The output was displayed as a series of pulse contours, synchronized with the heart-beat.

### 3.1.4 Protocol of ankle systolic blood pressure (ASBP), ankle-to-brachial systolic blood pressure index (ABI), toe systolic blood pressure (TSBP) and toe-to-brachial systolic blood pressure index (TBI) measurements

Smoking was not allowed for at least 2 hours prior to the start of the investigation. The following parameters were determined after the subjects had acclimatized and rested in the supine position for 15 minutes in a room with an ambient temperature between 22 and 25° C:

**1. Ankle systolic blood pressure (ASBP), expressed in mm Hg.**
A standard sized pneumatic cuff of 12 cm in width, as recommended by the American Heart Association, was used (23). After applying the cuff around the ankle and inflating it to a suprasystolic level, the ankle systolic blood pressure was determined by assessing accurately the pressure value at which the first audible pulsation was obtained during gradual cuff deflation.

**2. Ankle-to-brachial systolic blood pressure index (ABI).**
After wrapping the same pneumatic cuff around the arm and placing the Doppler probe over the brachial artery, the brachial systolic blood pressure (BSBP, expressed in mm Hg) was measured in the same way. The ratio of ASBP to BSBP was considered as ABI.

**3. Toe systolic blood pressure (TSBP), expressed in mm Hg.**
After a pneumatic cuff of 2 or 2.5 cm in width, depending on the length of the toe, was applied at the base of the first toe or the second toe in case of an amputation, the light-weighted photoplethysmograph was attached to the plantar side of that toe 22 with double-sided sticky tape. Then, after recording several pulsatile arterial waveforms, the cuff was inflated until the arterial blood flow was interrupted, as indicated by the disappearance of the pulsatile arterial waveforms. The pressure at which the pulsatile arterial waveforms did return during gradual cuff deflation was regarded as TSBP. If no pulsatile arterial waveforms were present before inflating the cuff, TSBP was taken to be zero mm Hg. Toe systolic blood pressure measurements were performed in the supine position (TSBP (supine)) and in the sitting position with the legs dependent after all subjects had acclimatized for 15 minutes in this position (TSBP (sitting)).

**4. Toe-to-brachial systolic blood pressure index (TBI).**
The ratios of TSBP (supine) and TSBP (sitting) to BSBP were defined as TBI (supine) and TBI (sitting), respectively. Since the brachial systolic blood pressure is not significantly influenced by a change in posture, BSBP, as assessed in the supine position, was used to calculate both TBI (supine) and TBI (sitting) (24,25).

## 3.2 RESULTS

### 3.2.1 Results of arteriography

Arteriography was performed in 62 patients. In 12 of these patients, however, the arteries below the knee could not be judged according to Morton. This was due to the fact that less than 4 arteries below the knee were visualized, because attention was focussed on the arteries above the knee and the distal popliteal artery (popliteal trifurcation). This resulted in 50 patients, whose arteriograms were available for this study. Twenty-two patients belonged to the F2-group and 28 patients belonged to the F3/4-group. For both groups the arteriographic classification, representing progressive degrees of macrocirculatory obstruction, is presented in Table 3.2.

**Table 3.2** Arteriographic classification for the F2-group and the F3/4-group, representing progressive degrees of macrocirculatory obstruction.

| ARTERIOGRAPHY-GROUP | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| F2 + F3/4 | n=50 | 2 | 20 | 2 | 5 | 5 | 16 |
| F2 | n=22 | 1 (4%) | 16 (73%) | 0 (0%) | 3 (14%) | 0 (0%) | 2 (9%) |
| F3/4 | n=28 | 1 (4%) | 4 (14%) | 2 (7%) | 2 (7%) | 5 (18%) | 14 (50%) |

F2 = F2-group, F3/4 = F3/4-group; n = number of patients.

Obstructed arteries above the knee (ARTERIOGRAPHY-GROUPS 2, 4 and 6) were seen in 21 out of the 22 patients of the F2-group (95%), whereas severe obstructions in the arteries below the knee (ARTERIOGRAPHY-GROUPS 5 and 6) were present in only 2 patients of this group (9%). In the F3/4-group, 20 out of 28 patients had obstructed arteries above the knee (ARTERIOGRAPHY-GROUPS 2, 4 and 6) (71%), whereas 19 patients had severe obstructions in the arteries below the knee (ARTERIOGRAPHY-GROUPS 5 and 6) (68%).

As expected, these findings indicate that in patients of the F2-group the obstructive process was mainly localized in the arteries above the knee, whereas in the majority of patients of the F3/4-group the arteries below the knee were also affected.

### 3.2.2 Results of ankle systolic blood pressure (ASBP) and ankle-to-brachial systolic blood pressure index (ABI) measurements in the supine position

The median ASBP of the control-group was 153 mm Hg (Figure 3.1). Patients of the F2-group and the F3/4-group had a median ASBP of 73 mm Hg and 60 mm Hg, respectively.

The ASBP values differed significantly between the 3 groups. Only one patient of the F2-group had an ASBP, which fell within the range of ASBP values of the control-group. A considerable overlap existed between the ranges of ASBP values of the F2-group and the F3/4-group.

The median ABI of the control-group, the F2-group and the F3/4-group was 1.15, 0.50 and 0.35, respectively (Figure 3.2).

A significant difference in ABI existed between the 3 groups. The ranges of ABI values of subjects of the control-group and patients with peripheral vascular

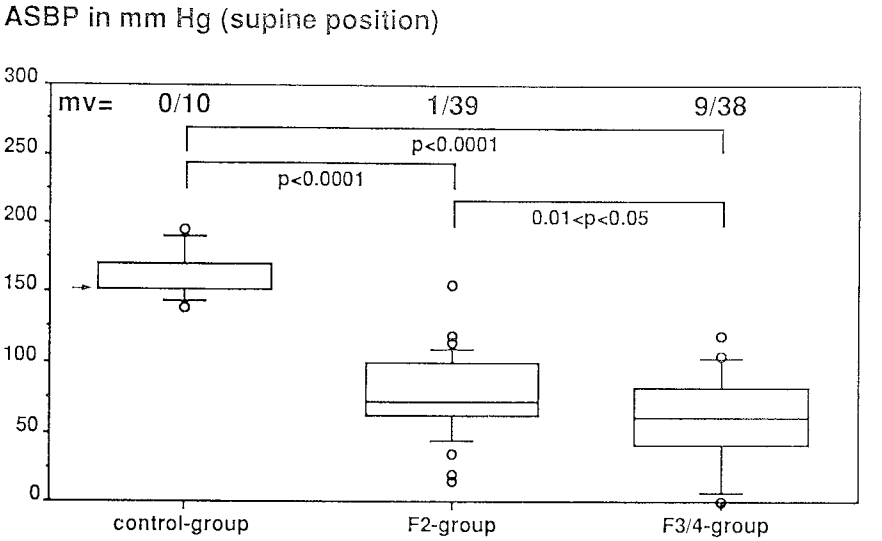


**Figure 3.1** Ankle systolic blood pressure (ASBP) in the supine position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s); → = median value.

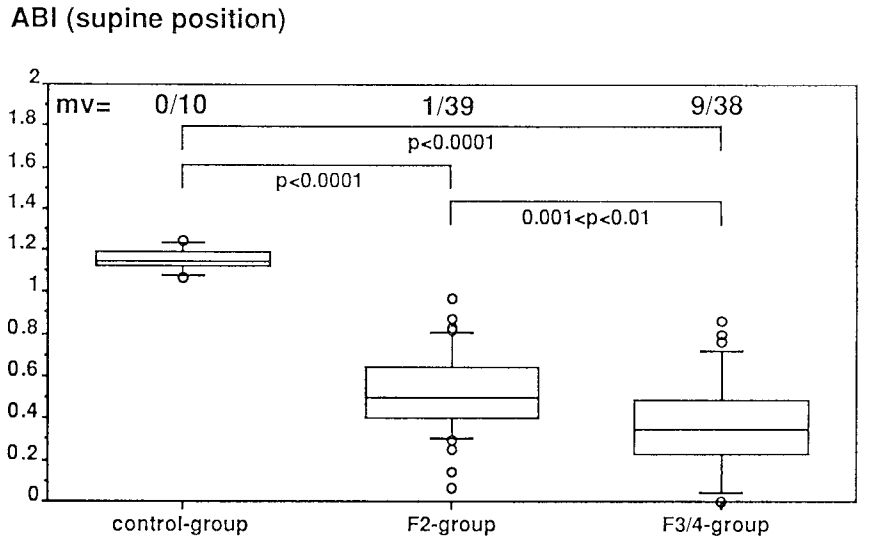


**Figure 3.2** Ankle-to-brachial systolic blood pressure index (ABI) in the supine position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s).

disease did not overlap, whereas the ranges of ABI values of the F2-group and the F3/4-group overlapped considerably.

In 1 patient of the F2-group and 9 patients of the F3/4-group no ASBP could be determined and hence no ABI could be calculated, because of incompressibility of the ankle arteries probably caused by arterial wall rigidity (Mönckeberg's sclerosis).

### 3.2.3 Results of toe systolic blood pressure (TSBP) and toe-to-brachial systolic blood pressure index (TBI) measurements in the supine and sitting position

**Supine position**

The median TSBP and TBI of subjects belonging to the control-group was 128 mm Hg and 0.89, respectively (Figures 3.3 and 3.4). Patients of the F2-group had a median TSBP of 35 mm Hg and a median TBI of 0.26. In patients of the F3/4-group the median TSBP and TBI was 0 mm Hg and 0.00, respectively.

Both the TSBP and TBI differed significantly between the 3 groups. TSBP and TBI values of respectively 6 and 4 patients of the F2-group fell within the range of the control-group. The ranges of TSBP and TBI values of the F2-group and the F3/4-group showed a considerable overlap.

**Sitting position**

The median TSBP and TBI of subjects of the control-group was 203 mm Hg and 1.49, respectively (Figures 3.5 and 3.6). Patients of the F2-group had a median TSBP of 143 mm Hg and a median TBI of 0.95. The median TSBP of patients belonging to the F3/4-group was 65 mm Hg, while their median TBI was 0.41.

A statistically significant difference in TSBP and TBI existed between the 3 groups. The ranges of TSBP and TBI values of healthy subjects and patients with moderate peripheral vascular disease (F2-group) overlapped considerably. The overlap between the ranges of TSBP values of the F2-group and the F3/4-group was small, as compared to the overlap between the ranges of TBI values of both groups.

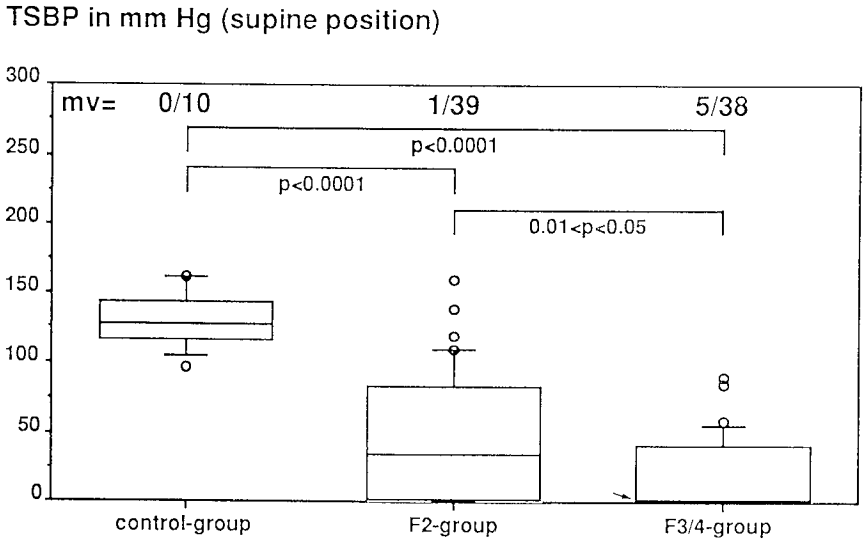


**Figure 3.3** Toe systolic blood pressure (TBSP) in the supine position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s); → = median value.

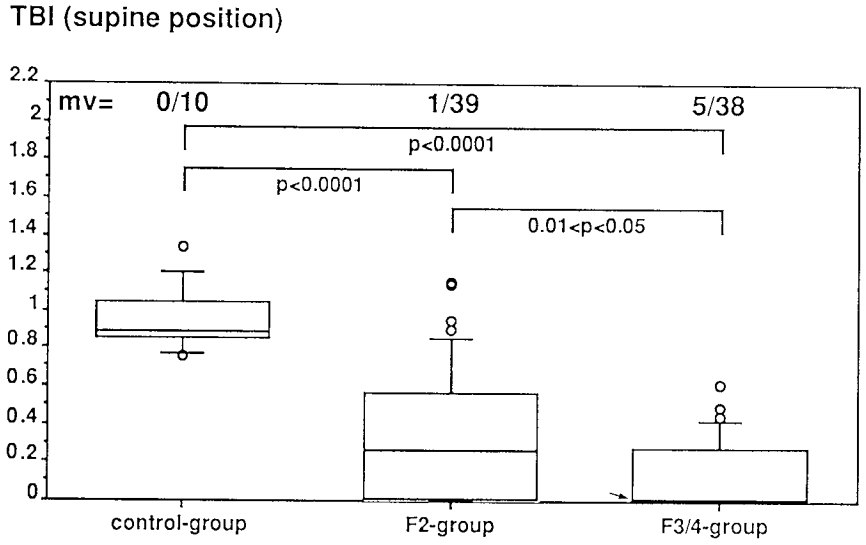


**Figure 3.4** Toe-to-brachial systolic blood pressure index (TBI) in the supine position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s); → = median value.

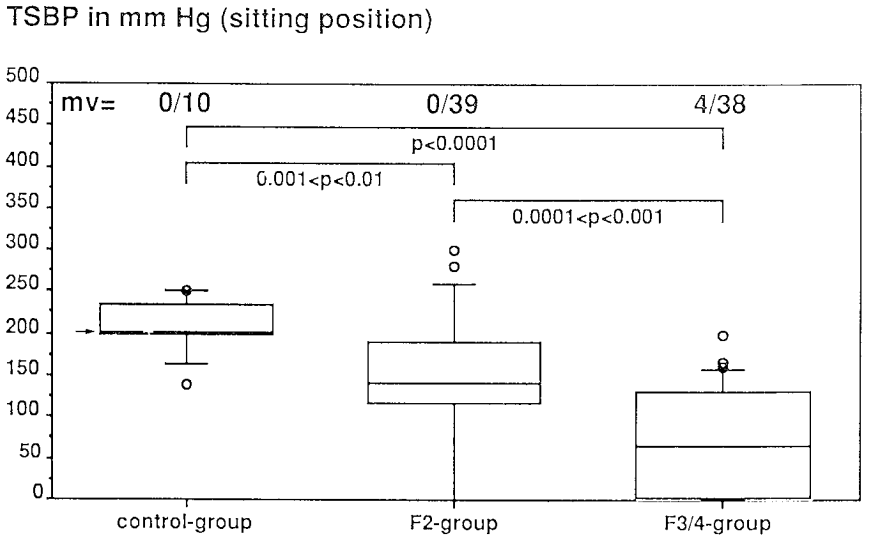


**Figure 3.5** Toe systolic blood pressure (TBSP) in the sitting position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s); → = median value.

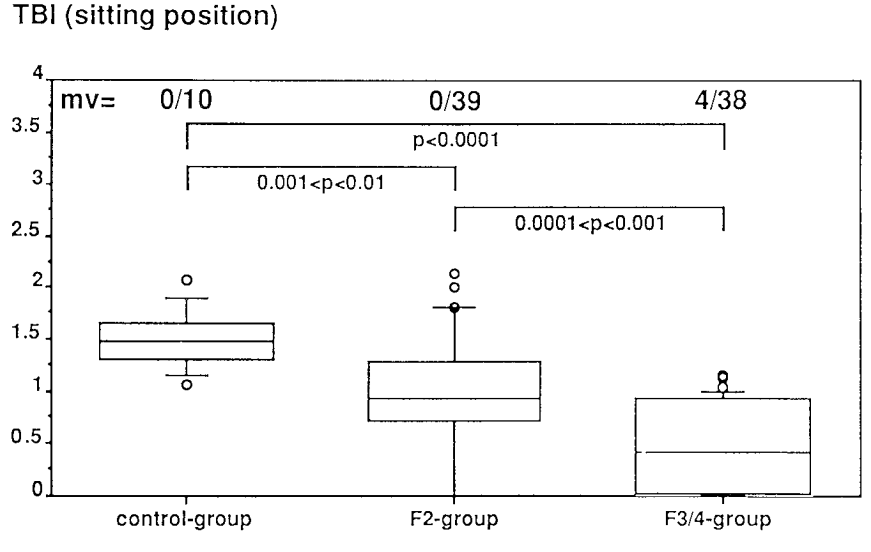


**Figure 3.6** Toe-to-brachial systolic blood pressure index (TBI) in the sitting position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s).

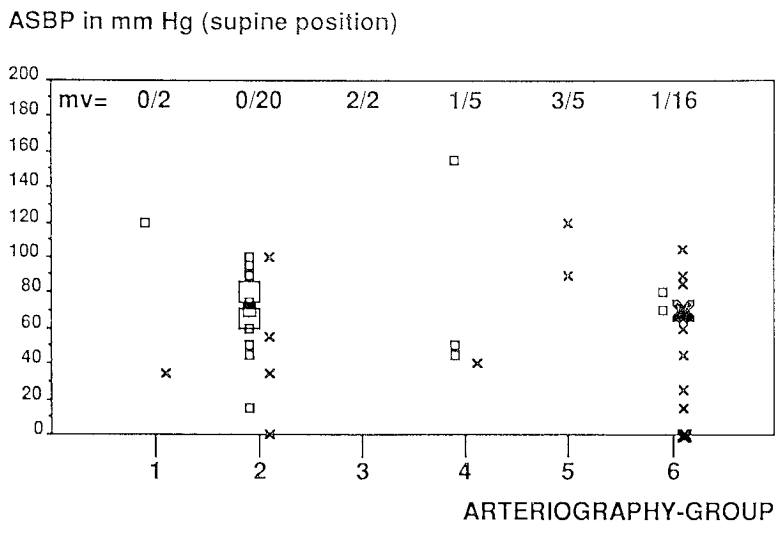


**Figure 3.7** Scatter plot of the ankle systolic blood pressure (ASBP) in the supine position for the various ARTERIOGRAPHY-GROUPS, representing progressive arteriographic degrees of macrocirculatory obstruction; □ = F2-group, × = F3/4-group. The size of the symbol corresponds to the number of data points that coincide at that location; abscissa: 1 = ARTERIOGRAPHY-GROUP 1, 2 = ARTERIOGRAPHY-GROUP 2, etc; mv = missing value(s).

### 3.2.4 Relation between ankle systolic blood pressure (ASBP), ankle-to-brachial systolic blood pressure index (ABI), toe systolic blood pressure (TSBP), toe-to-brachial systolic blood pressure index (TBI) and arteriography

In Figures 3.7-3.12, ASBP, ABI, TSBP and TBI are rearranged according to the corresponding ARTERIOGRAPHY-GROUPS, representing progressive arteriographic degrees of macrocirculatory obstruction. The absence of data for the ASBP and ABI in ARTERIOGRAPHY-GROUP 3 is explained by the presence of incompressible ankle arteries in both patients belonging to this ARTERIOGRAPHY-GROUP. ASBP and ABI values of the various ARTERIOGRAPHY-GROUPS showed a considerable overlap, as shown in Figures 3.7 and 3.8.

A considerable overlap also existed between TSBP and TBI values of the 6 ARTERIOGRAPHY-GROUPS in the supine as well as in the sitting position (Figures 3.9-3.12).

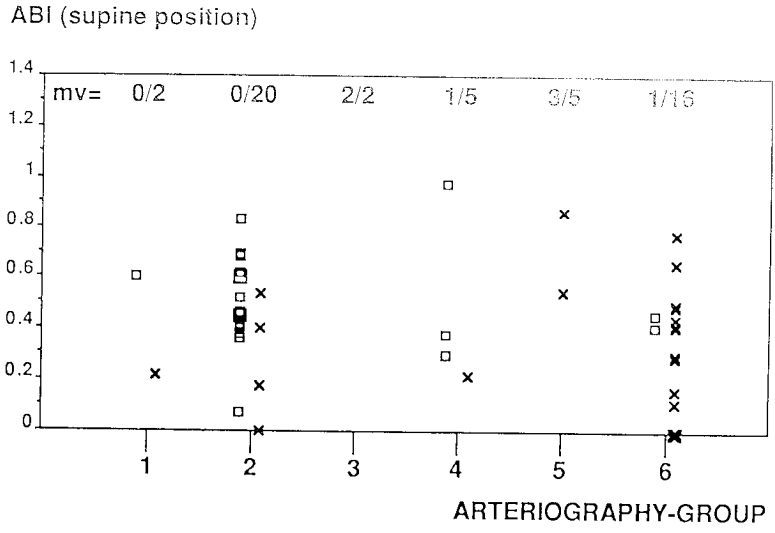


**Figure 3.8** Scatter plot of the ankle-to-brachial systolic blood pressure index (ABI) in the supine position for the various ARTERIOGRAPHY-GROUPS, representing progressive arteriographic degrees of macrocirculatory obstruction; □ = F2-group, × = F3/4-group. The size of the symbol corresponds to the number of data points that coincide at that location; abscissa: 1 = ARTERIOGRAPHY-GROUP 1, 2 = ARTERIOGRAPHY-GROUP 2, etc; mv = missing value(s).

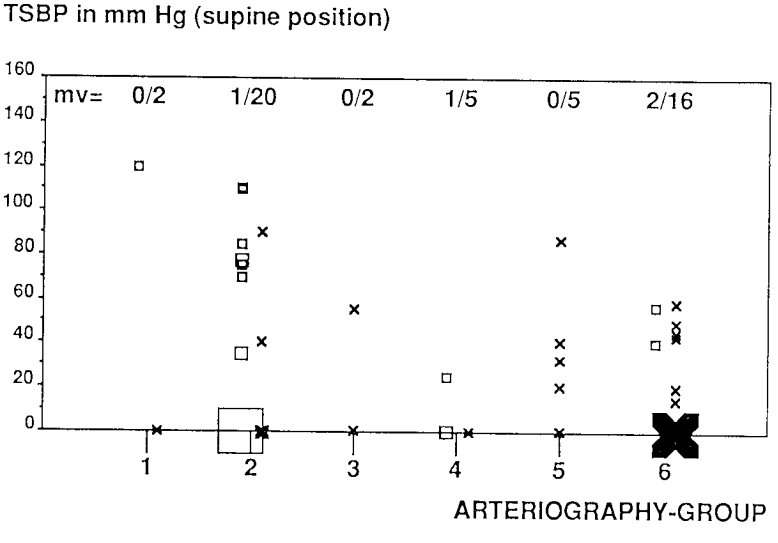


**Figure 3.9** Scatter plot of the toe systolic blood pressure (TSBP) in the supine position for the various ARTERIOGRAPHY-GROUPS, representing progressive arteriographic degrees of macrocirculatory obstruction; □ = F2-group, × = F3/4-group. The size of the symbol corresponds to the number of data points that coincide at that location; abscissa: 1 = ARTERIOGRAPHY-GROUP 1, 2 = ARTERIOGRAPHY-GROUP 2, etc; mv = missing value(s).

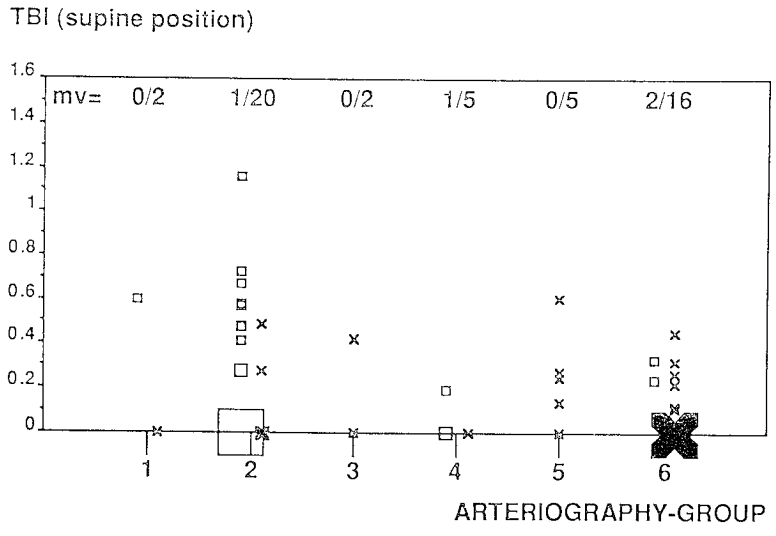


**Figure 3.10** Scatter plot of the toe-to-brachial systolic blood pressure index (TBI) in the supine position for the various ARTERIOGRAPHY-GROUPS, representing progressive arteriographic degrees of macrocirculatory obstruction; □ = F2-group, × = F3/4-group. The size of the symbol corresponds to the number of data points that coincide at that location; abscissa: 1 = ARTERIOGRAPHY-GROUP 1, 2 = ARTERIOGRAPHY-GROUP 2, etc; mv = missing value(s).

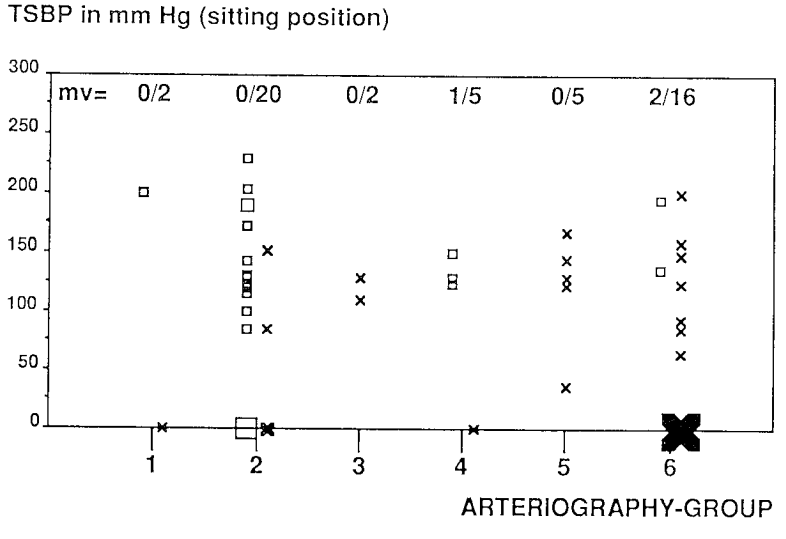


**Figure 3.11** Scatter plot of the toe systolic blood pressure (TSBP) in the sitting position for the various ARTERIOGRAPHY-GROUPS, representing progressive arteriographic degrees of macrocirculatory obstruction; □ = F2-group, × = F3/4-group. The size of the symbol corresponds to the number of data points that coincide at that location; abscissa: 1 = ARTERIOGRAPHY-GROUP 1, 2 = ARTERIOGRAPHY-GROUP 2, etc; mv = missing value(s).

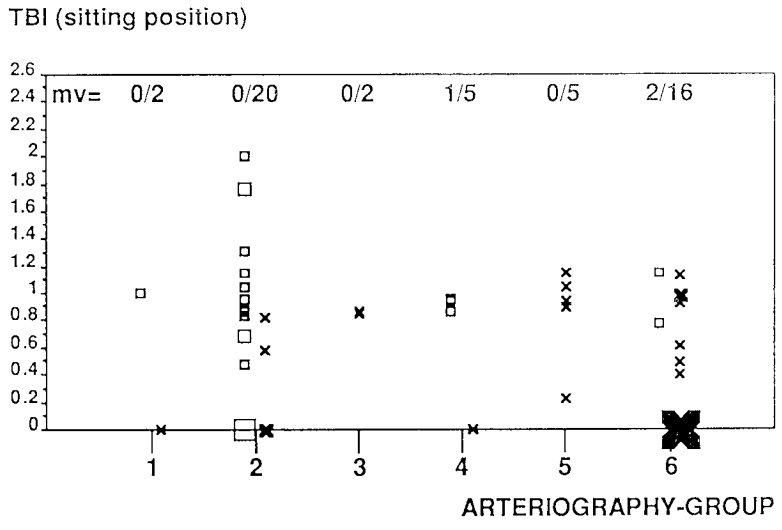


**Figure 3.12** Scatter plot of the toe-to-brachial systolic blood pressure index (TBI) in the sitting position for the various ARTERIOGRAPHY-GROUPS, representing progressive arteriographic degrees of macrocirculatory obstruction; □ = F2-group, × = F3/4-group. The size of the symbol corresponds to the number of data points that coincide at that location; abscissa: 1 = ARTERIOGRAPHY-GROUP 1, 2 = ARTERIOGRAPHY-GROUP 2, etc; mv = missing value(s).

## 3.3 DISCUSSION

A complete separation between healthy subjects on the one hand and patients with peripheral vascular disease (F2-group and F3/4-group) on the other can be made with the use of the ABI in the supine position. This, however, is not the case when the ASBP in the supine position, or the TSBP and TBI in the supine or sitting position are used. The progression from moderate (F2-group) to severe peripheral vascular disease (F3/4-group), however, is reflected in the ASBP, ABI, TSBP and TBI values of both patient groups, but the overlap between the two groups is substantial. The present results of ASBP and TSBP measurements in patients with severe peripheral vascular disease are in accordance with the observations of Carter, supporting his statement that the TSBP is a better indicator of severe ischaemia than the ASBP (20). When changing from the supine to the sitting position, the complete overlap between TSBP values of patients with moderate and severe peripheral vascular disease decreases to such an extent that the TSBP (sitting) provides a better estimation of the degree of peripheral vascular disease, as compared to ankle and toe systolic blood pressure measurements in the supine position.

ABI values above 0.80 in 4 patients with moderate peripheral vascular disease may be explained by the existence of well-developed collateral pathways (26). The high ASBP value of 155 mm Hg in 1 patient, which is in the range of ASBP values of healthy subjects, can probably be explained by the combination of well-developed collaterals and the high systemic systolic blood pressure of 160 mm Hg. Thirty-two patients with moderate peripheral vascular disease (84%) had a TSBP (supine) between 0 and 100 mm Hg, which is in agreement with the finding of Gundersen (27). In the remaining 6 patients, who had a TSBP (supine) above 100 mm Hg, the TSBP (supine) was higher than (n = 5) or equal to (n = 1) the ASBP, which indicates overestimation of the TSBP (supine). Since rigidity of digital arteries due to calcification is uncommon and seems not to interfere with TSBP measurements, the overestimated TSBP (supine) values probably resulted from the application of too small a cuff (26,28,29). In 4 of the 6 patients the overestimated values of TSBP (supine) caused the TBI (supine) to be within the range of healthy subjects.

In 9 patients with severe peripheral vascular disease (24%) the existence of arterial wall rigidity (Mönckeberg's sclerosis) interfered with ASBP measurements to such an extent that no ASBP could be determined. Eight of these 9 patients suffered from diabetes, which confirms that arterial wall stiffness is more likely to occur in diabetics (26). Four patients suffering from severe peripheral vascular disease had an ABI higher than 0.65 (0.66, 0.77, 0.80, 0.86), which was reported by Yao to be the upper limit (16). The high ABI values in these 4 patients, 3 of whom had diabetes, are probably caused by overestimation of their ASBP, due to the presence of arterial wall rigidity. Three of these 4 patients (2 diabetics, 1 non-diabetic), of whom the arteries below the knee could be reviewed arteriographically, showed a Morton-stage 3 of outflow obstruction (ARTERIOGRAPHY-GROUPS 5 (n = 1) and 6 (n = 2), indeed suggesting the presence of arterial wall rigidity. Twenty-four per cent of the patients with severe ischaemia (n = 8) had a TSBP (supine) above 40 mm Hg, which is in agreement with the observations of Gundersen, who reported a percentage of 18 with this value (27). Since 7 of these 8 patients had a TBI (supine) below 0.50 (range: 0.22- 0.49), their high TSBP (supine) is probably caused by a relatively high systemic systolic blood pressure.

The arteriographic degree of macrocirculatory obstruction does not correspond to ASBP and ABI values or to TSBP and TBI values in the supine and sitting position, as indicated by the considerable overlap between the different ARTERIOGRAPHY-GROUPS. The tendency of the TBI (supine) to decrease in patients with a more severe degree of arterial obstruction is in accordance with the finding of Lezack and Carter (30). They reported a correlation between the TBI, as measured in the supine position, and the severity of the arteriographic lesions, although also in their study a considerable overlap was shown.

## 3.4 CONCLUSION

It can be concluded that the TSBP, and especially the TSBP in the sitting position, is the most appropriate variable to estimate the degree of peripheral vascular disease. When performing TSBP measurements, however, the possibility of over-estimation due to the application of too small a cuff has to be taken into account, especially if pressure values at the ankle level appear to be lower.

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# CHAPTER 4

# SKIN MICROCIRCULATION

In this chapter the investigation of the total and nutritional skin microcirculation with the use of transcutaneous oxygen pressure ($tcpO_2$) monitoring and intravital skin capillary microscopy, respectively, is described.

## 4.1 INVESTIGATIVE TECHNIQUES

### 4.1.1 $TcpO_2$ monitoring

Gerlach was probably the first to show the absorption of oxygen through the intact human skin in 1851 by measuring chemically a reduction in the amount of oxygen 24 hours after he had tied a horse bladder, filled with air, firmly to his chest (1).

The membrane-covered polarographic oxygen electrode, constructed by Clark in 1956, made it possible to measure oxygen pressure ($pO_2$) routinely (2). Measurements of the $pO_2$ at the skin surface with the use of such a membrane-covered platinum electrode, as performed by Evans and Taylor in 1967, showed $pO_2$ values at the surface of the non-hyperaemized adult skin to be close to zero mm Hg (3). Higher $tcpO_2$ values as a result of a better oxygenation of the skin were obtained by local skin heating, causing skin hyperaemia due to vasodilation of skin blood vessels and a shift of the oxyhaemoglobin curve to the right (4,5).

In healthy newborns a close relation was found between arterial oxygen pressure ($paO_2$) values and $tcpO_2$ values, as measured at an electrode core temperature of 43° C (6). This close relation was not only observed in healthy neonates, but also in sick infants with mild hypotension, hypothermia or anaemia, indicating the general applicability of the method (7). Subsequently, $tcpO_2$ measurements have become the method of choice to monitor $paO_2$ continuously and non-invasively in newborn infants. In adults $tcpO_2$ monitoring can be applied to perceive the development of hypoxia and shock (8).

The first $tcpO_2$ measurements to estimate oxygen delivery to the skin in patients with arterial obstructive disease of the lower limb were probably performed by Tønnesen in 1978 (9). He reported zero $tcpO_2$ values at rest at 44° C in patients with moderate and severe rest pain (stage 3 according to Fontaine), although skin isotope clearance indicated the presence of some blood supply to the skin. Furthermore, it has been shown that during the inhalation of 100% of oxygen the rate of rise of $tcpO_2$ was significantly reduced and the maximum level of $tcpO_2$ was considerably lower in patients with severe peripheral vascular disease (stages 3 and 4 according to Fontaine) (10,11). Despite considerable overlap, a statistically significant difference in $tcpO_2$ at rest at 44-45° C has been shown to be present between healthy subjects and patients with intermittent claudication (stage 2 according to Fontaine) (12-14). At 44-45° C, the $tcpO_2$ response to the release of a transient arterial occlusion and to exercise, however, showed a more significant difference with a considerably smaller overlap (13-15).

A severely impaired oxygen supply to the skin often results in ischaemic skin necrosis, which finally may lead to amputation. The optimum level of amputation can be selected by assessing skin viability with the use of $tcpO_2$ measurements. A $tcpO_2$ value at rest of 40 mm Hg or more at the site of amputation (electrode core temperature 44-45° C) generally leads to successful healing of the stump (16,17). Since treatment of patients with peripheral vascular disease intends to restore perfusion and hence oxygen supply to the skin, $tcpO_2$ measurements are suitable for the evaluation of therapy (18-21).

## Methods

In the present study $tcpO_2$ was measured polarographically. This method is based on the reduction of oxygen at a platinum cathode when a voltage is applied between this cathode and an anode. The following electrochemical reaction occurs when a platinum cathode and a silver anode are used:

At the cathode: $O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$
At the anode: $4Ag \rightarrow 4Ag^+ + 4e^-$.

The resulting electrical current is proportional to the oxygen tension at the skin surface. Although oxygen electrodes consume oxygen, the amount consumed can practically be ignored when cathodes with a very small diameter are used.

Two TCM2 TC OXYGEN MONITORS (Radiometer, Copenhagen), each connected to a $tcpO_2$ electrode, were used in this study. Both $tcpO_2$ electrodes combine in one and the same unit: a Clark-type oxygen electrode and a heating element combined with a temperature sensor to ensure a constant electrode core temperature of 44° C. The Clark-type oxygen electrodes consist of a platinum

cathode surrounded by a silver anode. A microcomputer system converts the currents, generated by the two $tcpO_2$ electrodes, into $pO_2$ values, expressed in mm Hg (range: 0-999 mm Hg). Since zero-current drift was minimal and stability good, only a single-point calibration procedure before the start of every new measurement had to be carried out. Furthermore, both $tcpO_2$ electrodes were remembranized every two weeks. Data were recorded with the use of a TCM200 TC RECORDER at a chart speed of 0.5 cm/min. $TcpO_2$ measurements were performed at an intact part of the skin at the dorsum of the first metatarsophalangeal joint of the foot of interest. In addition, a reference $tcpO_2$ value was determined in the left subclavian region of the chest, which in general is well perfused, to be informed of the systemic level of skin oxygen delivery. Before securing the electrodes to the patient, the skin at the selected measuring sites was shaved, if necessary, and degreased with alcohol. Then fixation rings were placed on these measuring sites. Before firmly mounting both electrodes a contact liquid was put on the skin inside these rings. The probe cables were secured to the skin with surgical tape at appropriate distance from the measuring sites.

### 4.1.2 Intravital skin capillary microscopy

The first observations of capillaries and the red blood cells inside them were made in animals by the Dutch microscopist van Leeuwenhoek (1632-1723) and the Italian anatomist Malphigi (1628-1694) in the 17th century (22,23). In human beings the microcirculation was probably investigated for the first time by the famous Dutch physician Boerhaave (1668-1738) at the level of the bulbar conjunctiva (22). The development of new biomicroscopic techniques allowed a more detailed visualization of the human microvessels. A microscope with incident illumination was used by Hueter in 1879 to investigate the capillaries of the human lip (24). Beautiful pictures of skin capillaries in the back of the hand and the finger nailfold were obtained by Lombard in 1912 by improving the transparency of the skin with a thin layer of glycerine or a transparent oil (25). Intravital skin capillary microscopy was also used to measure capillary blood pressure. Instead of observing colour changes when pressure was applied to the skin, capillary blood pressure was measured by taking as criterion the sudden sharp forward acceleration of corpuscles after lowering the pressure on the underlying skin, which had caused reversed corpuscular flow (26). The first photographic pictures of human capillaries were probably made by Weiß in 1916 (23). He constructed a device, that enabled him to examine and photograph capillaries in any part of the human skin. He reported that in patients with arteriosclerosis capillaries were elongated and narrowed and that the capillaries became more tortuous and blood velocity lower with increasing severity of arteriosclerosis. In his book 'Die Kapillaren der menschlichen Körperoberfläche in gesunden und kranken Tagen', published in 1922, Müller summarized the most

important findings of his pupils Weiß, Niehau, Parrisius and Heimberger (27). The time course of microcirculatory events in human capillaries, like the motion of corpuscles, was assessed by means of cinematography by Crawford and Rosenberger in 1926 (28). In the fifties and sixties intravital skin capillary microscopy was especially applied in dermatological practice (29). The morphology of capillaries in a variety of diseases was described by Davis and Landau in their atlas 'Clinical capillary microscopy', published in 1966 (22). The diagnostic value of morphological intravital skin capillary microscopy, however, was doubted by Ryan (30).

Structural changes of skin capillaries at the medial part of the lower limb and at the dorsum of the foot in patients with arterial obstructive disease of the lower limb have been studied by Fagrell with the use of intravital skin capillary microscopy (24,31,32). He noted a discrepancy between the macrocirculation and the nutritional skin microcirculation. In some patients a marked decrease in toe systolic blood pressure was not associated with structural changes of capillaries.

The first measurements of red blood cell velocity were probably performed by van Leeuwenhoek in 1674. He measured a red blood cell velocity of approximately 2 mm/s in capillaries of tadpole tails using the dimensions of a grain of sand as the unit of distance and the time, necessary to pronounce a four-syllable word, as the unit of time (33). In 1974, Bollinger and colleagues introduced a television microscopy technique and made sequential measurements of the red blood cell velocity in human nailfold capillaries possible (34). This technique allowed them to evaluate red blood cell velocity patterns and hence capillary haemodynamics in various clinical conditions by measuring the displacement of plasma gaps from successive video frames. Since that time, capillary haemodynamics and morphology have been studied in patients, especially in those with vasospastic and ischaemic hand phenomena (35-39). Because (red) blood cell velocities at rest showed a considerable variation, they could hardly be used for comparative studies between different subjects (40). The response to provocations, such as a period of ischaemia, caused by inflating a pneumatic cuff to a suprasystolic level, however, led to quite different responses in patients and healthy subjects (40-44). It has become a routine in microcirculatory research to apply provocations pertinent to the disease investigated.

## Methods

Toe nailfold capillaries were investigated using an intravital skin capillary microscope (modified Leitz Orthoplan). To minimize disturbing reflections and to increase the transparency of the skin, a drop of paraffin oil was applied to the area of interest of the toe. Incident illumination was performed using a 100 Watt mercury vapour lamp (Calflex heat reflection filter; heat absorption filter; DC-power supply), an incident fluorescence illuminator (Leitz Ploemopak 2.1; mag-

nification 1.25x), and a Leitz POL-cube (45). This Leitz POL-cube consists of a polarizer, a 50% mirror, positioned at 45° to the optical axis, and a crossed analyzer. Images were directly projected onto a TV camera (Philips, Type LDH 0400/01; newvicon XQ 1275, 2/3 inch tube), that could be rotated with respect to the optical axis of the system, and were displayed on a TV monitor (Philips, Type 2122/01). For off-line analysis the images were stored on tape via a videocassette recorder (Sony Betamax, model No. SL-C9ES). A Leitz objective L4x (numerical aperture = 0.14) was used for density measurements, while a Leitz objective L10 × (numerical aperture = 0.30) was used for velocity and diameter measurements. The final optical magnification was 5× and 12.5×, respectively. The calculated interline spacing on the monitor was 5 and 2 μm, respectively.

### 4.1.3 Protocol of $tcpO_2$ monitoring and intravital skin capillary microscopy

#### Protocol of $tcpO_2$ monitoring

After all subjects had acclimatized and rested in the supine position for 15 minutes in a room where the ambient temperature was kept constant between 22 and 25° C, $tcpO_2$ monitoring was started. Smoking was not permitted for at least a 2 hour period previous to the start of the examination. A recording period of about 10-15 minutes was necessary before simultaneously a stable baseline $tcpO_2$ value from the dorsum of the first metatarsophalangeal joint of the foot and a stable reference $tcpO_2$ value from the chest were obtained at rest. After determining the reference $tcpO_2$ value at the chest, $tcpO_2$ monitoring at this site was stopped and the $tcpO_2$ electrode was disconnected. Subsequently, all subjects were asked to inhale calmly 100% of oxygen through a mask, that was connected to an oxygen cylinder. Oxygen was supplied at a rate of 10 litres/min. About 10 minutes of oxygen inhalation were necessary to reach a maximum $tcpO_2$ value, which remained stable during further oxygen inhalation. After reaching this maximum $tcpO_2$ value, all subjects started to breathe ambient air again. When the $tcpO_2$ at the foot had returned to the baseline value at rest, perfusion of the foot was stopped by rapidly inflating to a suprasystolic level a 12 cm wide pneumatic cuff, wrapped around the ankle. Cuff pressure was kept at that level for up to 3-5 minutes, depending on the pain tolerance of the subject. Occlusion caused $tcpO_2$ to decrease to about 0 mm Hg in almost all subjects. Subsequently, the $tcpO_2$ response after rapidly deflating the cuff was recorded. The following parameters were derived from the readings:

**1. RESTING $TCPO_2$, expressed in mm Hg.**

The stable baseline $tcpO_2$ value at rest, determined at an intact part of the skin at the dorsum of the first metatarsophalangeal joint of the foot, after a recording period of 10-15 minutes. RESTING $TCPO_2$ reflects total skin blood flow at rest.

**2. CHEST $TCPO_2$, expressed in mm Hg.**
The stable reference $tcpO_2$ value at rest, obtained in the left subclavian region of the chest, after a recording period of 10-15 minutes.

**3. RATE $TCPO_2$, expressed in mm Hg/min.**
The maximum rate of rise of $tcpO_2$ at the foot during oxygen inhalation, usually occurring in the initial phase.

**4. PEAK $TCPO_2$, expressed in mm Hg.**
The maximum $tcpO_2$ value at the foot during oxygen inhalation.

**5. T50% $TCPO_2$, expressed in seconds (s).**
The time needed to reach 50% of the RESTING $TCPO_2$ following the release of a transient arterial occlusion (3-5 minutes). T50% $TCPO_2$ indirectly provides information about the degree of arteriolar dilation at rest.

To investigate the influence of the change in posture on the results of $tcpO_2$ monitoring, all measurements at the foot were subsequently repeated with the subjects in a comfortable sitting position with the legs dependent. These measurements were performed after an adaptation period of at least 15 minutes.

**Protocol of intravital skin capillary microscopy**
Following $tcpO_2$ monitoring, subjects were seated with the legs dependent on a chair with hydraulically adjustable height to position the foot on the stage of the microscope. To obtain a stable image, the leg of interest was gently immobilized at knee level without obstructing blood flow to the foot. The forefoot itself was fixed in a mass of clay. In this position subjects rested for 15 minutes. Since red blood cell velocity at rest often varies rhythmically, images of capillaries were recorded for about 2 minutes (46). Capillary loops were recorded with the arteriolar limbs parallel to the video lines. After this 'period of rest', blood flow to the foot was arrested by inflating a pneumatic cuff (width 12 cm), which was wrapped around the ankle, to a suprasystolic pressure. After a 1 minute occlusion, the cuff was rapidly deflated. The subsequent reactive hyperaemia images were stored on videotape. Images of 3 to 5 different capillaries, randomly chosen in the nailfold of the first toe, were recorded in this way. In 2 patients, however, images of capillaries at the dorsum of the first metatarsophalangeal joint of the foot were recorded since their first and second toe had been amputated. Red blood cell velocity was measured off-line with the use of the video flying spot method (47-49). Four spots, generated on the video screen and moving along the video lines, were synchronized with the moving red blood cells and plasma gaps. The following parameters were determined from the video images:

**1. Red blood cell velocity at rest (RBCV), expressed in mm/s.**
Red blood cell velocity at rest was measured in 4 to 5 different periods of about 20 seconds during the 'period of rest'and mean red blood cell velocity at rest was calculated for each capillary. Mean RBCV for that particular subject was obtained by averaging the mean red blood cell velocities at rest of 3 to 5 different capillaries.

**2. Peak red blood cell velocity (PRBCV), expressed in mm/s.**
By repetitively replaying the response following the release of a 1 minute arterial occlusion, the highest red blood cell velocity in the capillary under investigation was assessed with the use of the flying spot device. This velocity was regarded as peak red blood cell velocity for that particular capillary. Mean PRBCV of a subject was calculated by averaging the peak red blood cell velocities, determined in 3 to 5 capillaries.

**3. Red blood cell velocity index (RBCVI).**
By dividing the peak red blood cell velocity by the mean red blood cell velocity at rest of the same capillary, RBCVI of that specific capillary was calculated. Mean RBCVI for a subject was determined by averaging the different calculated red blood cell velocity indices, obtained from each of the capillaries examined.

**4. Time-to-peak red blood cell velocity (TTP), expressed in seconds (s).**
Time-to-peak red blood cell velocity is the time that elapsed between the release of the cuff and the moment of peak red blood cell velocity. Mean TTP for that subject was calculated by averaging the time-to-peaks of 3 to 5 investigated capillaries.

PRBCV, RBCVI and TTP have in common that they indirectly give information about the degree of arteriolar dilation at rest.

**5. Diameter of the arteriolar limb of the capillary loop (DIAMETER), expressed in μm.**
The flying spot device was also used to measure from still images the diameters of the arteriolar limbs of the same capillary loops, in which the aforementioned parameters had been determined (Figure 4.1). A spot was positioned over the first visible part of the arteriolar limb of the capillary loop. By adjusting the width of the spot to the width of the capillary, the arteriolar limb diameter at that position was determined. A correction was made if the edges of spot and arteriolar limb were not exactly parallel. This procedure was repeated 2 to 3 times along the same arteriolar limb at equal intervals from the first measurement. The length of these intervals, which depended on the length of the arteriolar limb investigated, ranged

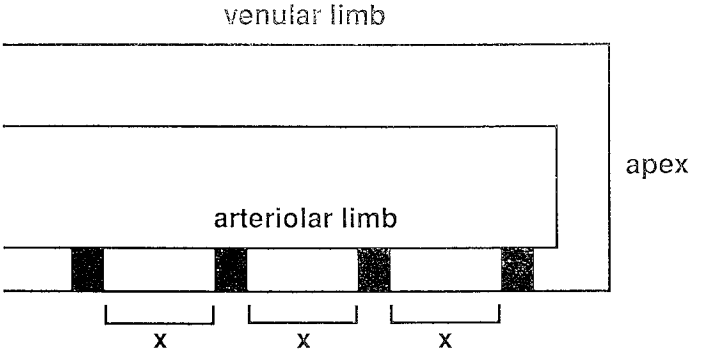


**Figure 4.1** Drawing of a capillary loop with arteriolar and venular limb, and apex. Four spots are successively positioned over the arteriolar limb at equal intervals (×) and adjusted to the width of the arteriolar limb.

from appproximately 11 to about 22 μm. The mean diameter of the arteriolar limb of that capillary was calculated as the mean of these measurements. Mean DIAMETER for a subject was obtained by averaging the mean diameters of all investigated capillaries.

### 6. Density of toe nailfold capillaries (DENSITY), expressed in n (number)/mm$^2$.

The number of capillary loops, identified as filled with red blood cells, was counted from still images on the full size of the TV monitor. At each count the apexes of the capillaries of the distal row were situated close to the right hand boundary of the monitor. In every subject at least 3 screens, each covering a different part of the same nailfold, were counted. The resulting mean number of capillary loops per screen was converted to a DENSITY value per mm$^2$ by dividing it by the investigated skin surface area of 1.86 mm$^2$.

### 7. Nutritional skin blood flow at rest (NSBF), expressed in mm$^3$/s per mm$^2$ of the investigated skin surface area.

The nutritional skin blood flow at rest per mm$^2$ of the investigated skin surface area was calculated by multiplying the mean capillary blood flow at rest ($BF_{cap}$) by the number of capillaries per mm$^2$ (DENSITY). For each capillary investigated, $BF_{cap}$ was calculated using the formula:

$$\pi \cdot \left(\frac{D_{cap}}{2}\right)^2 \cdot V_{cap},$$

where Dcap is the mean diameter of the arteriolar limb of a capillary loop, expressed in mm, and Vcap is the mean red blood cell velocity at rest of that same capillary, expressed in mm/s.

## 4.2 RESULTS

### 4.2.1 Results of $tcpO_2$ monitoring in the supine and sitting position

**Supine position**

The median stable baseline $tcpO_2$ at rest at the dorsum of the first metatarsophalangeal joint of the foot (RESTING $TCPO_2$) in subjects of the control-group was 57 mm Hg (Figure 4.2). In patients of the F2-group the median RESTING $TCPO_2$ was 46 mm Hg and in patients of the F3/4-group 4 mm Hg. RESTING $TCPO_2$ was significantly different between the 3 groups.

The median stable reference $tcpO_2$ at rest at the chest (CHEST $TCPO_2$) was 63 mm Hg in the control-group, whereas in the F2-group the median CHEST $TCPO_2$

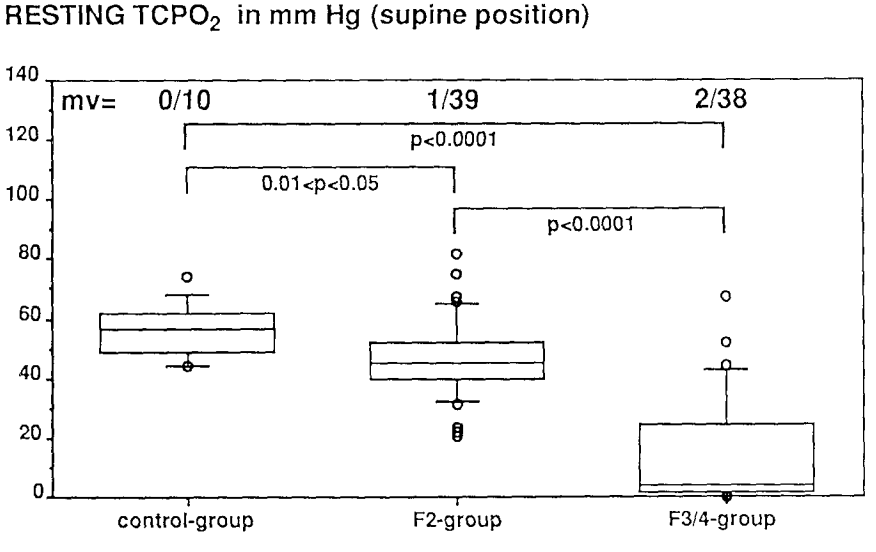


**Figure 4.2** Stable baseline $tcpO_2$ at rest at the dorsum of the first metatarsophalangeal joint of the foot (RESTING TCPO2) in the supine position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s).

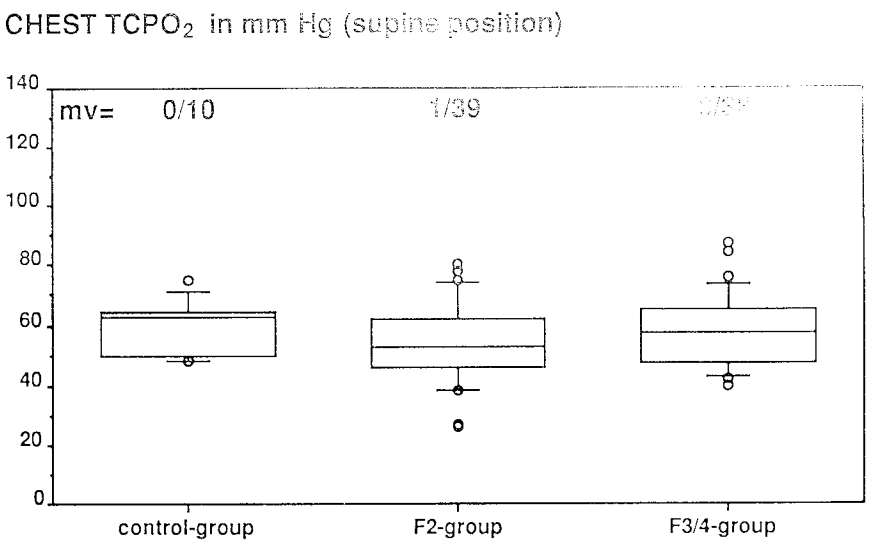


**Figure 4.3** Stable reference $tcpO_2$ at rest at the chest (CHEST $TCPO_2$) in the supine position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s).

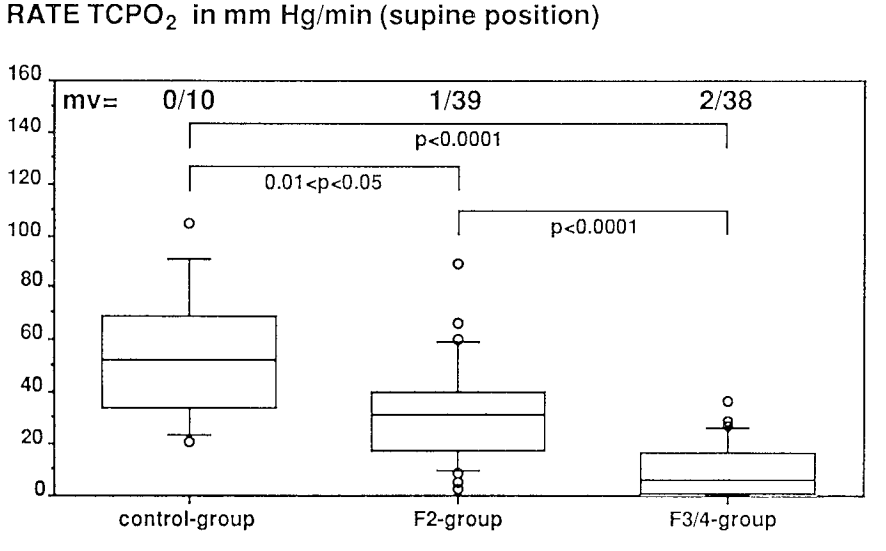


**Figure 4.4** Maximum rate of rise of $tcpO_2$ at the dorsum of the first metatarsophalangeal joint of the foot during oxygen inhalation (RATE $TCPO_2$) in the supine position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s).

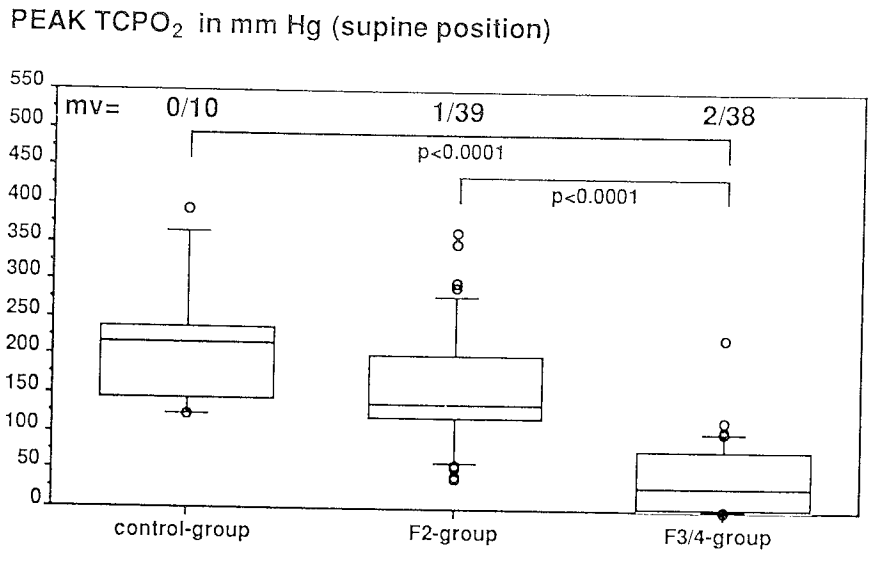


**Figure 4.5** Maximum level of $tcpO_2$ at the dorsum of the first metatarsophalangeal joint of the foot during oxygen inhalation (PEAK $TCPO_2$) in the supine position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s).

was 53 mm Hg (Figure 4.3). The F3/4-group had a median CHEST $TCPO_2$ of 57 mm Hg. CHEST $TCPO_2$ did not differ significantly between the 3 groups.

During oxygen inhalation the median maximum rate of rise of $tcpO_2$ at the dorsum of the first metatarsophalangeal joint of the foot (RATE $TCPO_2$) was 52 mm Hg/min, 32 mm Hg/min and 7 mm Hg/min in the control-group, the F2-group and the F3/4-group, respectively (Figure 4.4). An increase in the severity of lower limb ischaemia was associated with a significant decrease in RATE $TCPO_2$.

The maximum level of $tcpO_2$ at the dorsum of the first metatarsophalangeal joint of the foot during oxygen inhalation (PEAK $TCPO_2$) had a median value of 217 mm Hg in subjects of the control-group, while patients of the F2-group and the F3/4-group had a median PEAK $TCPO_2$ of 138 mm Hg and 30 mm Hg, respectively (Figure 4.5). PEAK $TCPO_2$ in the F3/4-group was significantly lower than in the control-group and the F2-group.

The median time needed to reach 50% of the RESTING $TCPO_2$ at the dorsum of the first metatarsophalangeal joint of the foot following the release of a transient arterial occlusion (T50% $TCPO_2$) was 45 s in the control-group (Figure 4.6). The median T50% $TCPO_2$ in patients of the F2-group and the F3/4-group was 72 s and 114 s, respectively. T50% $TCPO_2$ was significantly prolonged with increasing severity of peripheral vascular disease.

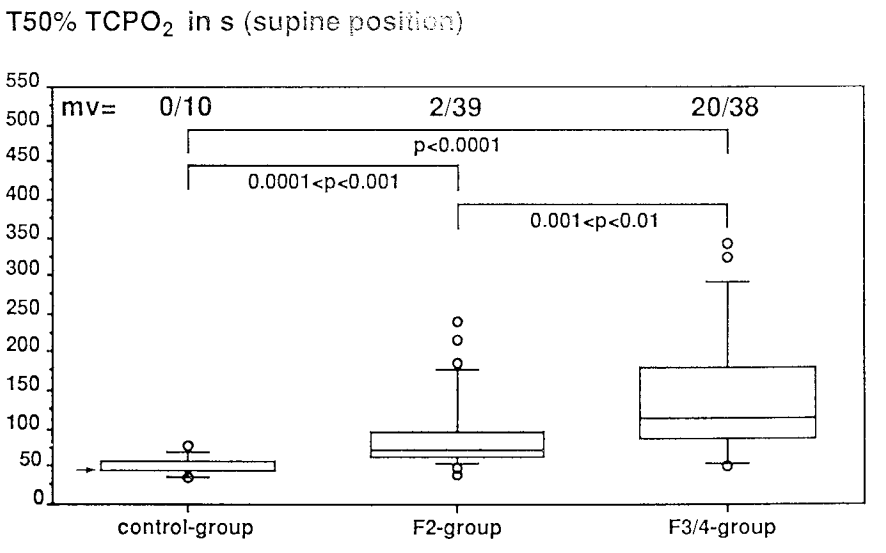


**Figure 4.6** Time needed to reach 50% of the RESTING $TCPO_2$ at the dorsum of the first metatarsophalangeal joint of the foot following the release of a transient arterial occlusion (T50% $TCPO_2$)in the supine position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s); → = median value.

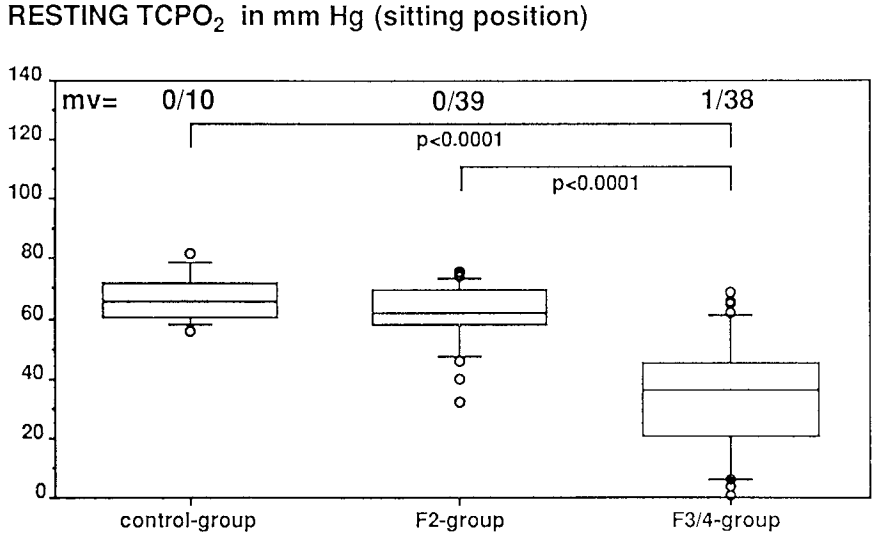


**Figure 4.7** Stable baseline $tcpO_2$ at rest at the dorsum of the first metatarsophalangeal joint of the foot (RESTING $TCPO_2$) in the sitting position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s).

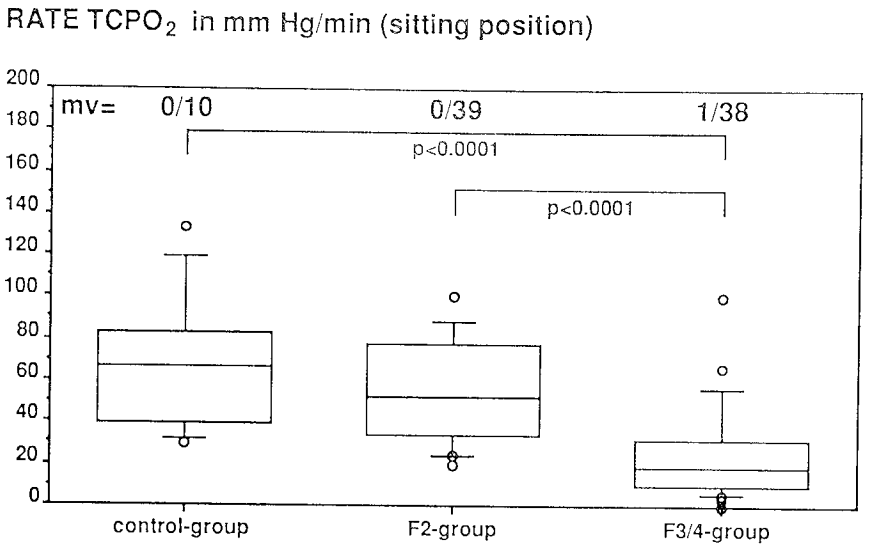


**Figure 4.8** Maximum rate of rise of $tcpO_2$ at the dorsum of the first metatarsophalangeal joint of the foot during oxygen inhalation (RATE $TCPO_2$) in the sitting position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s).

## Sitting position

The median stable baseline $tcpO_2$ at rest at the dorsum of the first metatarsophalangeal joint of the foot (RESTING $TCPO_2$) in subjects of the control-group, the F2-group and the F3/4-group was 66 mm Hg, 62 mm Hg and 36 mm Hg, respectively (Figure 4.7). Patients of the F3/4-group had a significantly lower RESTING $TCPO_2$, as compared to subjects of the control-group and the F2-group.

During oxygen inhalation the median value of the maximum rate of rise of $tcpO_2$ at the dorsum of the first metatarsophalangeal joint of the foot (RATE $TCPO_2$) was 67 mm Hg/min in the control-group (Figure 4.8). Patients of the F2-group had a median RATE $TCPO_2$ of 53 mm Hg/min, while patients of the F3/4-group had a median RATE $TCPO_2$ of 19 mm Hg/min. A significant difference in RATE $TCPO_2$ was only present between the F3/4-group on the one hand and the control-group and the F2-group on the other.

The maximum level of $TCPO_2$ at the dorsum of the first metatarsophalangeal joint of the foot during oxygen inhalation (PEAK $TCPO_2$) had a median value of 230 mm Hg, 199 mm Hg and 81 mm Hg in subjects of the control-group, the F2-group and the F3/4-group, respectively (Figure 4.9). PEAK $TCPO_2$ of the

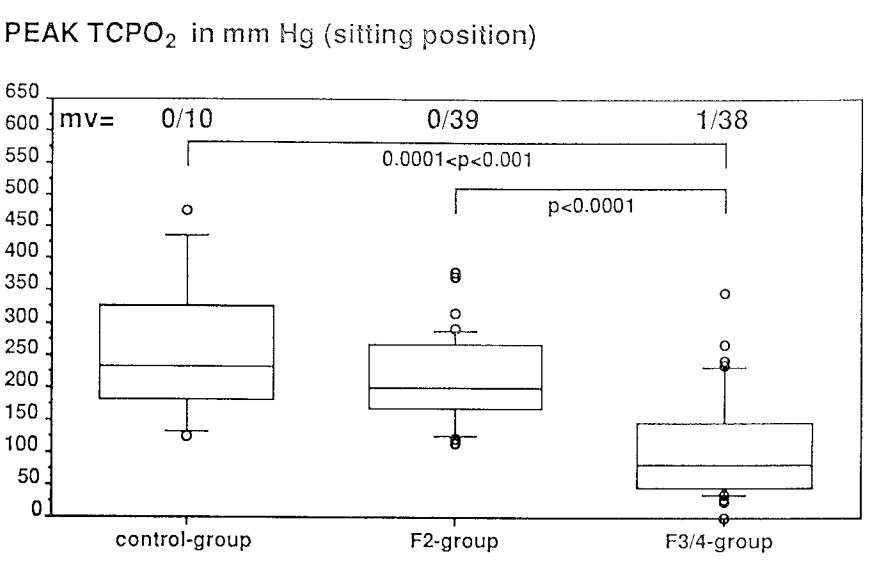


**Figure 4.9** Maximum level of $tcpO_2$ at the dorsum of the first metatarsophalangeal joint of the foot during oxygen inhalation (PEAK $TCPO_2$) in the sitting position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s).

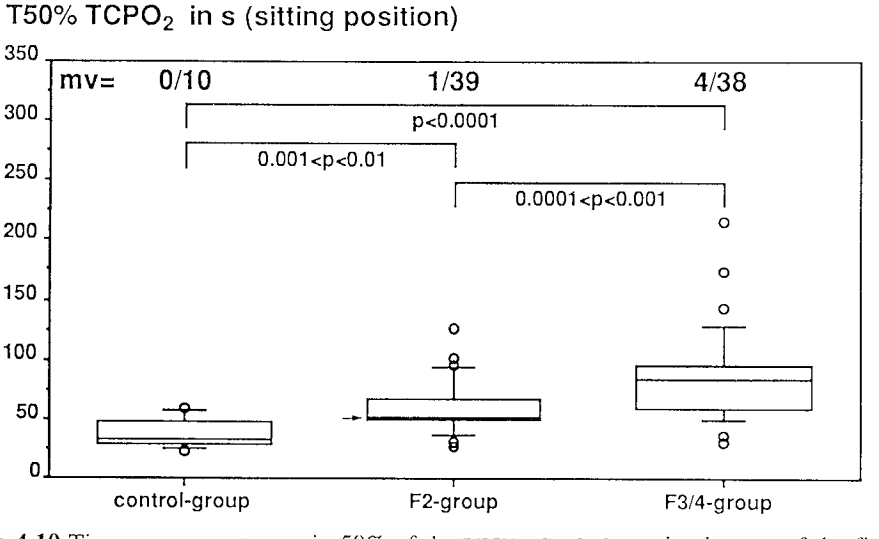


**Figure 4.10** Time necessary to regain 50% of the RESTING $TCPO_2$ at the dorsum of the first metatarsophalangeal joint of the foot following the release of a transient arterial occlusion (T50% $TCPO_2$) in the sitting position in healthy subjects (control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2-group and F3/4-group), as shown by box plots; mv = missing value(s); → = median value.

F3/4-group was significantly lower, as compared to the control-group and the F2-group.

The median time necessary to regain 50% of the RESTING $TCPO_2$ at the dorsum of the first metatarsophalangeal joint of the foot following the release of a transient arterial occlusion (T50% $TCPO_2$) was 32 s, 51 s and 84 s in the control-group, the F2-group and the F3/4-group, respectively (Figure 4.10). Progression of peripheral vascular disease was associated with a significantly prolonged T50% $TCPO_2$.

As shown in Table 4.1, changing from the supine to the sitting position resulted in a significant improvement in RESTING $TCPO_2$, RATE $TCPO_2$ and T50% $TCPO_2$ in all 3 groups investigated. PEAK $TCPO_2$, however, was only significantly increased in the F2-group and the F3/4-group.

**Table 4.1** Influence of posture (supine versus sitting) on $tcpO_2$ parameters at the foot. Median values are presented.

| | CONTROL | F2 | F3/4 |
|---|---|---|---|
| RESTING $TCPO_2$ supine (mm Hg) | 57 | 46 | 4 |
| | ** | **** | **** |
| RESTING $TCPO_2$ sitting (mm Hg) | 66 | 62 | 36 |
| RATE $TCPO_2$ supine (mm Hg/min) | 52 | 32 | 7 |
| | * | **** | **** |
| RATE $TCPO_2$ sitting (mm Hg/min) | 67 | 53 | 19 |
| PEAK $TCPO_2$ supine (mm Hg) | 217 | 138 | 30 |
| | ns | **** | **** |
| PEAK $TCPO_2$ sitting (mm Hg) | 230 | 199 | 81 |
| T50% $TCPO_2$ supine (s) | 45 | 72 | 114 |
| | ** | **** | ** |
| T50% $TCPO_2$ sitting (s) | 32 | 51 | 84 |

RESTING $TCPO_2$ = stable baseline $TCPO_2$ at rest at the foot, RATE $TCPO_2$ = maximum rate of rise of $TCPO_2$ at the foot during oxygen inhalation, PEAK $TCPO_2$ = maximum level of $tcpO_2$ at the foot during oxygen inhalation, T50% $TCPO_2$ = time needed to reach 50% of the RESTING $TCPO_2$ at the foot following the release of a transient arterial occlusion; CONTROL = control-group, F2 = F2-group, F3/4 = F3/4-group; Levels of significance: * = $0.01<p<0.05$, ** = $0.001<p<0.01$, *** = $0.0001<p<0.001$, **** = $p<0.0001$, ns = not significant.

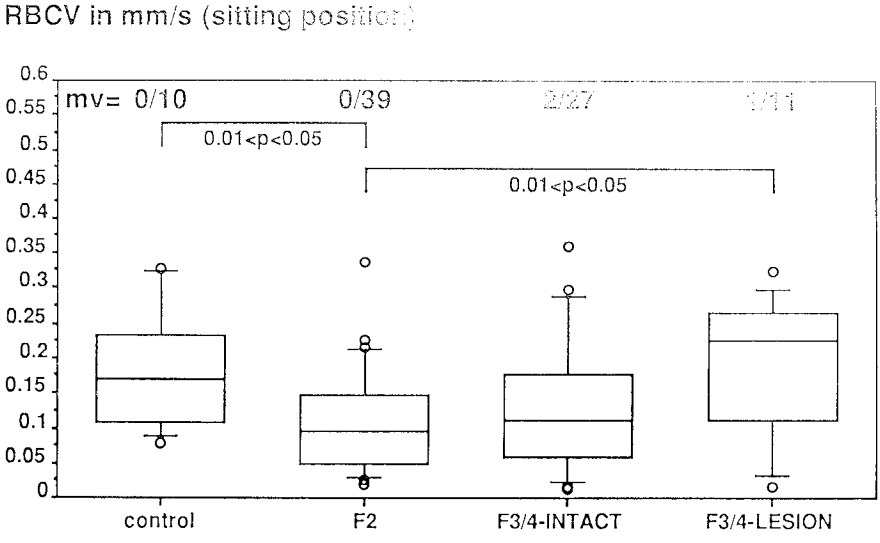


**Figure 4.11** Red blood cell velocity at rest (RBCV) in the sitting position in healthy subjects (control = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2 = F2-group, F3/4-INTACT = F3/4-INTACT-group and F3/4-LESION = F3/4-LESION-group), as shown by box plots; mv = missing value(s).

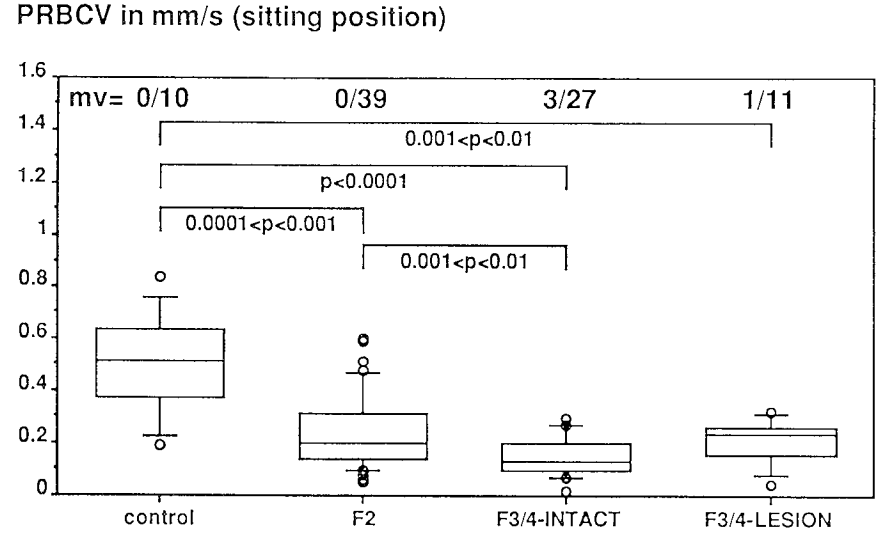


**Figure 4.12** Peak red blood cell velocity following the release of a transient arterial occlusion (PRBCV) in the sitting position in healthy subjects (control = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2 = F2-group, F3/4-INTACT = F3/4-INTACT-group and F3/4-LESION = F3/4-LESION-group), as shown by box plots; mv = missing value(s).

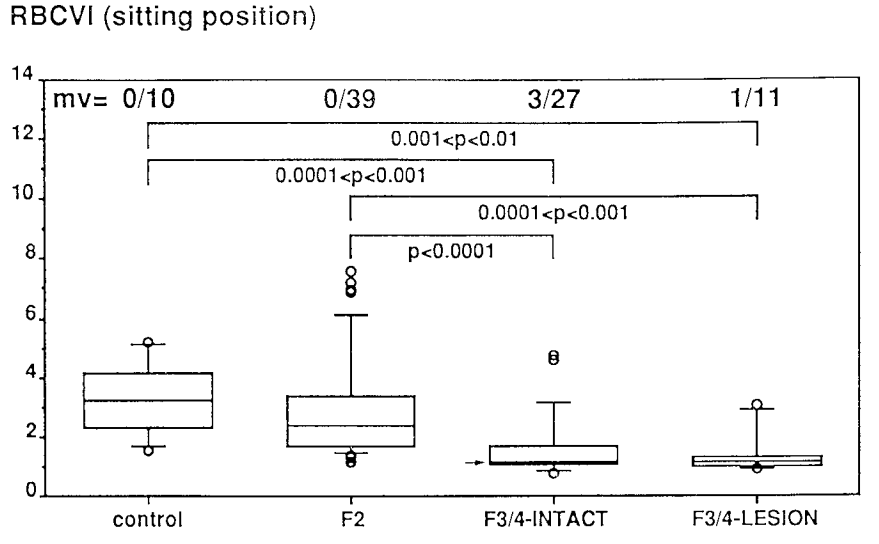


**Figure 4.13** Ratio of PRBCV to RBCV (RBCVI (red blood cell velocity index)) in the sitting position in healthy subjects (control = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2 = F2-group, F3/4-INTACT = F3/4-INTACT-group and F3/4-LESION = F3/4-LESION-group), as shown by box plots; mv = missing value(s); → = median value.

### 4.2.2 Results of intravital skin capillary microscopy in the sitting position

The median red blood cell velocity at rest (RBCV) in the control-group was 0.171 mm/s and in patients of the F2-group 0.095 mm/s (Figure 4.11). Patients of the F3/4-INTACT-group, of whom an intact part of the skin was investigated, had a median RBCV of 0.110 mm/s. In patients of the F3/4-LESION-group, on whom intravital skin capillary microscopy was performed in the rim of an ischaemic skin lesion, a median RBCV of 0.227 mm/s was found. RBCV of the F2-group was significantly lower, as compared to the control-group and the F3/4-LESION-group.

The median peak red blood cell velocity following the release of a transient arterial occlusion (PRBCV) was 0.519 mm/s and 0.205 mm/s in the control-group and the F2-group, respectively (Figure 4.12). In patients of the F3/4-INTACT-group and the F3/4-LESION-group (rim of ischaemic skin lesion) the median PRBCV was 0.128 mm/s and 0.237 mm/s, respectively. A significantly lower PRBCV was found in patients with peripheral vascular disease, as compared to healthy subjects.

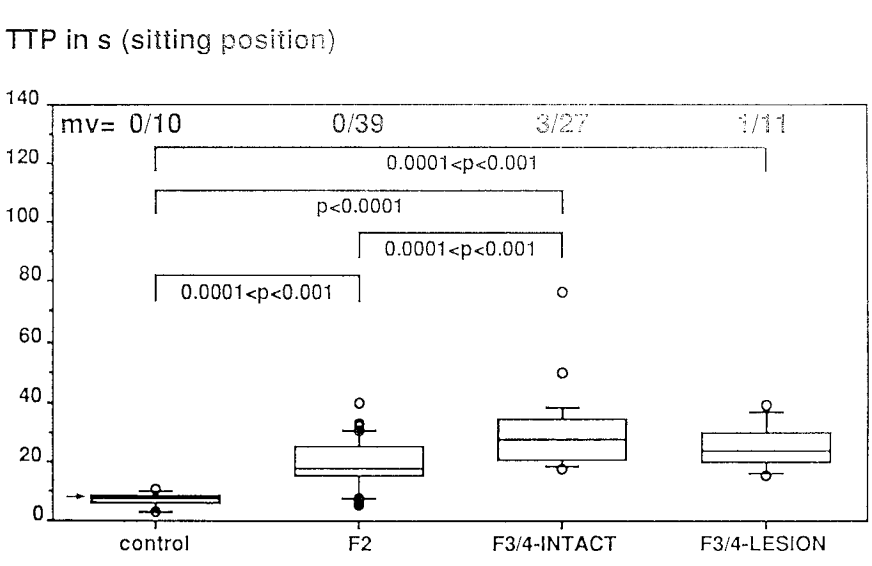


**Figure 4.14** Time-to-peak red blood cell velocity following the release of a transient arterial occlusion (TTP) in the sitting position in healthy subjects (control = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2 = F2-group, F3/4-INTACT = F3/4-INTACT-group and F3/4-LESION = F3/4-LESION-group), as shown by box plots; mv = missing value(s); → = median value.

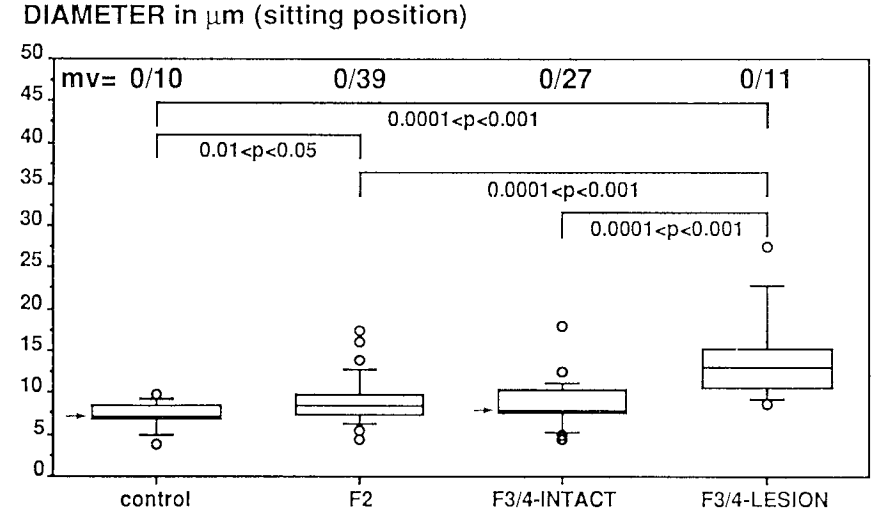


**Figure 4.15** Diameter of the arteriolar limbs of capillary loops (DIAMETER) in the sitting position in healthy subjects (control = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2 = F2-group, F3/4-INTACT = F3/4-INTACT-group and F3/4-LESION = F3/4-LESION-group), as shown by box plots; mv = missing value(s); → = median value.

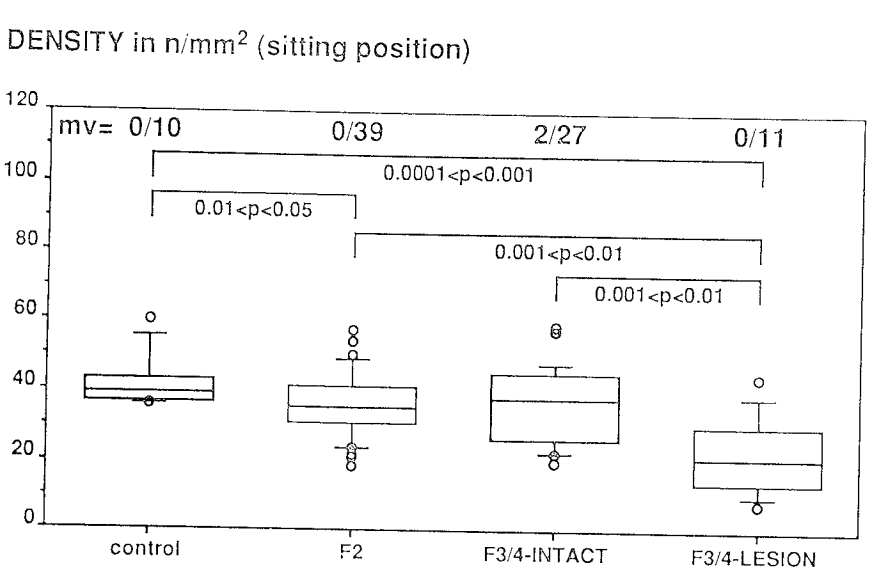


**Figure 4.16** Number of blood-filled capillaries per $mm^2$ of the investigated skin surface area (DENSITY) in the sitting position in healthy subjects (control = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2 = F2-group, F3/4-INTACT = F3/4-INTACT-group and F3/4-LESION = F3/4-LESION-group), as shown by box plots; mv = missing value(s).

The median ratio of PRBCV to RBCV (RBCVI (red blood cell velocity index)) was 3.24 in the control-group, 2.42 in the F2-group, 1.17 in the F3/4-INTACT-group and 1.18 in the F3/4-LESION-group (rim of ischaemic skin lesion) (Figure 4.13). The RBCVI of the F3/4-INTACT-group and the F3/4-LESION-group were similar and both significantly lower, as compared to the control-group and the F2-group.

The median time-to-peak red blood cell velocity following the release of a transient arterial occlusion (TTP) was 8 s and 17 s in the control-group and the F2-group, respectively (Figure 4.14). In patients of the F3/4-INTACT-group and the F3/4-LESION-group (rim of ischaemic skin lesion) the median TTP was 28 s and 24 s, respectively. Patients with peripheral vascular disease had a significantly prolonged TTP, as compared to healthy subjects.

The median diameter of the arteriolar limbs of capillary loops (DIAMETER) of the control-group was 7 μm, while in the F2-group a median DIAMETER of 9 μm was found (Figure 4.15). The F3/4-INTACT-group had a median DIAMETER of 8 μm. In the F3/4-LESION-group (rim of ischaemic skin lesion) the median DIAMETER was 13 μm. The DIAMETER was significantly larger in rims of ischaemic skin lesions (F3/4-LESION-group), as compared to intact parts of the skin in patients with equally severe peripheral vascular disease (F3/4-INTACT-group).

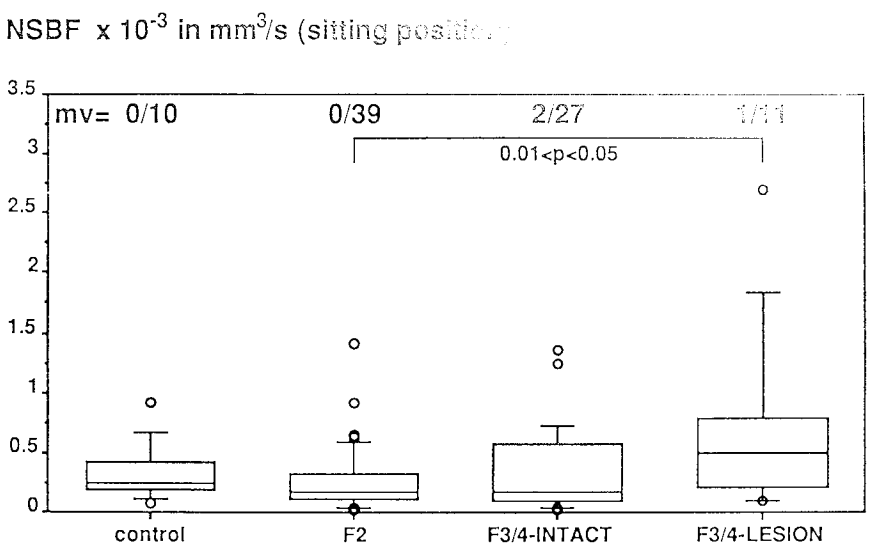


**Figure 4.17** Nutritional skin blood flow at rest per $mm^2$ of the investigated skin surface area (NSBF) in the sitting position in healthy subjects (control = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (F2 = F2-group, F3/4-INTACT = F3/4-INTACT-group and F3/4-LESION = F3/4-LESION-group), as shown by box plots; mv = missing value(s).

The median number of blood-filled capillaries per mm2 of the investigated skin surface area (DENSITY) was $39/mm^2$ in the control-group and $35/mm^2$ in the F2-group (Figure 4.16). In the F3/4-INTACT-group the median DENSITY was $38/mm^2$ and in the F3/4-LESION-group (rim of ischaemic skin lesion) $21/mm^2$. Patients of the F3/4-LESION-group had a significantly lower DENSITY, as compared to healthy subjects, patients of the F2-group and patients of the F3/4-INTACT-group.

The median nutritional skin blood flow at rest per $mm^2$ of the investigated skin surface area (NSBF) was $0.252 \cdot 10^{-3}$ $mm^3/s$ in subjects of the control-group (Figure 4.17). Patients of the F2-group and the F3/4-INTACT-group had a median NSBF of $0.176 \cdot 10^{-3}$ $mm^3/s$ and $0.166 \cdot 10^{-3}$ $mm^3/s$, respectively. In patients of the F3/4-LESION-group (rim of ischaemic skin lesion) the median NSBF was $0.490 \cdot 10^{-3}$ $mm^3/s$. The NSBF of the F3/4-LESION-group did not differ significantly from the NSBF of the F3/4-INTACT-group despite a markedly higher median NSBF.

## 4.3 DISCUSSION

In the supine position skin oxygen delivery at rest, which reflects total skin blood flow at rest, is slightly decreased in patients with moderate peripheral vascular

disease (intermittent claudication), as compared to healthy subjects. In patients with severe peripheral vascular disease (rest pain, ischaemic skin lesion(s)), however, oxygen supply to the skin at rest and hence total skin blood flow at rest is significantly impaired, as compared to both healthy subjects and patients with moderate peripheral vascular disease. The absence of a statistically significant difference in skin oxygen delivery at rest at the chest between the 3 groups excludes a contribution of ventilation or haemoglobin properties to the observed differences in peripheral skin oxygen supply. Furthermore, the inhalation of 100% of oxygen causes a proportional increase in skin oxygen delivery in the 3 groups investigated, preserving the aforementioned differences. It emphasizes that in patients with various degrees of lower limb ischaemia the perfusion is the limiting factor as far as peripheral oxygen supply to the skin is concerned. The response to the release of a transient arterial occlusion provides a far better separation between healthy subjects and patients with moderate peripheral vascular disease, as compared to the skin oxygen delivery at rest and during oxygen inhalation. This is in accordance with the findings of other investigators (14,50,51).

Changing from the supine to the sitting position with the legs dependent leads to a moderate but unexpected increase in skin oxygen delivery at rest in healthy subjects and patients with moderate peripheral vascular disease, abolishing the small difference in total skin blood flow at rest between both groups. This increase in total skin blood flow at rest can probably be explained by the absence of vasoconstrictive mechanisms, caused by heating the skin (52-55). Under normal conditions these vasoconstrictive mechanisms cause a reduction in lower limb blood supply upon leg dependency in healthy subjects and patients with moderate peripheral vascular disease (52-54,56-59). Although a considerable improvement in total skin blood flow at rest upon changing from the supine to the sitting position is seen in patients with severe lower limb ischaemia, it still differs significantly from healthy subjects and patients with moderate peripheral vascular disease.

Investigation of the nutritional skin microcirculation shows that in patients with severe peripheral vascular disease the nutritional skin blood flow at rest in the intact skin is maintained at a normal level. The increase in red blood cell velocity following the release of a transient arterial occlusion in these patients, however, is reduced to a minimum, indicating the dilation of the arterioles at rest to be almost at the maximum. This considerable dilation at the arteriolar level may probably be regarded as a precapillary adjustment to increase nutritional skin blood flow at rest. Since in the intact skin of patients with severe peripheral vascular disease arteriolar dilation at rest is almost at the maximum, a further increase in ischaemia, due to progression of the disease and/or external skin compression, for example, caused by pinching shoes, may result in a crucial

reduction in the number of blood-filled capillaries and hence the surface area for nutrition. This, finally, may cause the development of ischaemic skin lesions.

Examination of the nutritional skin microcirculation in the rims of ischaemic skin lesions, which indicate a more disturbed nutritional skin microcirculation, indeed reveals a significantly smaller number of capillaries, as compared to patients with the same degree of peripheral vascular disease, of whom intact skin parts are investigated microscopically. Despite this small number of capillaries, nutritional skin blood flow at rest in the rims of ischaemic skin lesions is similar to the nutritional skin blood flow at rest in the intact skin. This is likely to be due to the significant increase in the diameter of the arteriolar limbs of capillary loops, probably in response to metabolites released from these damaged skin parts. This increase in capillary diameter in the rims of ischaemic skin lesions may be regarded as an ultimate possibility to increase nutritional skin blood flow at rest in response to a further increase in local ischaemia. The morphological changes, dilation of capillaries and diminished capillary density, as observed in the rims of ischaemic skin lesions, are in accordance with the findings of Fagrell (24).

Investigations with the use of intravital skin capillary microscopy also show that the response to the release of a transient arterial occlusion provides the best distinction between healthy subjects, patients with moderate and patients with severe peripheral vascular disease. This finding confirms the statement of Fagrell and colleagues that the post-occlusive reactive hyperaemia response is of great value in nutritional skin microcirculatory research (40-43).

## 4.4 CONCLUSION

Nutritional skin blood flow at rest in the intact skin of patients with severe peripheral vascular disease is preserved in the sitting position. The maintenance of nutritional skin blood flow at rest likely results from dilation of arterioles. When this precapillary adjustment is almost at the maximum, progression of the disease and external skin compression probably account for an insufficient nutritional skin microcirculation, ultimately leading to the development of ischaemic skin lesions. The increase in the diameter of the arteriolar limbs of capillary loops, seen in the rims of ischaemic skin lesions, may be caused by metabolites, released from these ischaemic skin lesions. This increase in capillary diameter may be considered as an ultimate possibility to increase nutritional skin blood flow at rest.

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# CHAPTER 5

# RELATION BETWEEN THE MACROCIRCULATION AND THE SKIN MICROCIRCULATION

In this chapter the relation between the macrocirculation, as examined with the use of toe systolic blood pressure (TSBP) and arteriography, and the skin microcirculation, as investigated with the use of $tcpO_2$ monitoring and intravital skin capillary microscopy, is presented. The reasons for preferring TSBP to ankle systolic blood pressure to provide information about the condition of the macrocirculation, have been given in chapter 4. The relation between the results of $tcpO_2$ and TSBP measurements in the sitting position is not discussed in this chapter, because these measurements were performed under different experimental conditions. $TcpO_2$ was monitored during local skin heating (electrode core temperature 44° C), which overrules vasoconstrictive mechanisms normally elicited upon leg dependency in healthy subjects and patients with moderate peripheral vascular disease, while TSBP measurements were performed without heating the skin (1-8).

## 5.1 RELATION BETWEEN THE SKIN MICROCIRCULATION AND TOE SYSTOLIC BLOOD PRESSURE (TSBP)

The relation between skin oxygen delivery at rest (RESTING $TCPO_2$), which reflects total skin blood flow at rest, and TSBP in the supine position in patients with moderate and severe peripheral vascular disease (F2-group and F3/4-group, respectively) and healthy subjects (control-group), is visualized in Figure 5.1.

The absence of a significant difference between RESTING $TCPO_2$ values of patients with peripheral vascular disease (range: 35-82 mm Hg) and healthy subjects (indicated by $\Delta$ in Figure 5.1), who have a TSBP higher than 60 mm Hg,

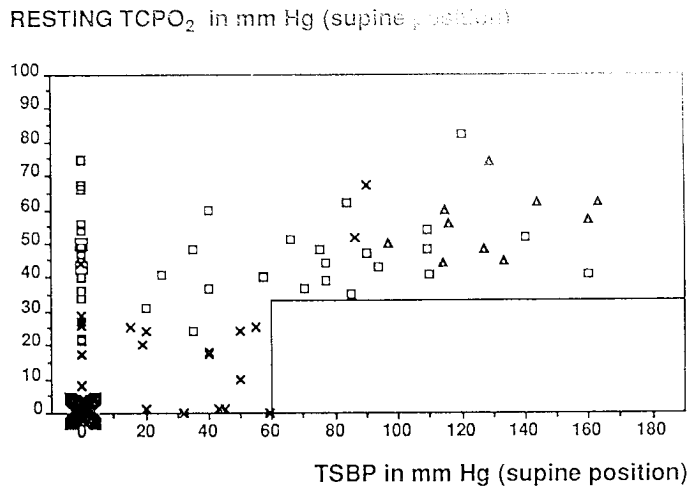


**Figure 5.1** Stable baseline $tcpO_2$ at rest at the dorsum of the first metatarsophalangeal joint of the foot (RESTING $TCPO_2$) in the supine position for toe systolic blood pressure (TSBP) in the supine position in healthy subjects ( Δ = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (□ = F2-group and × = F3/4-group. The size of the symbol corresponds to the number of data points that coincide at that location.

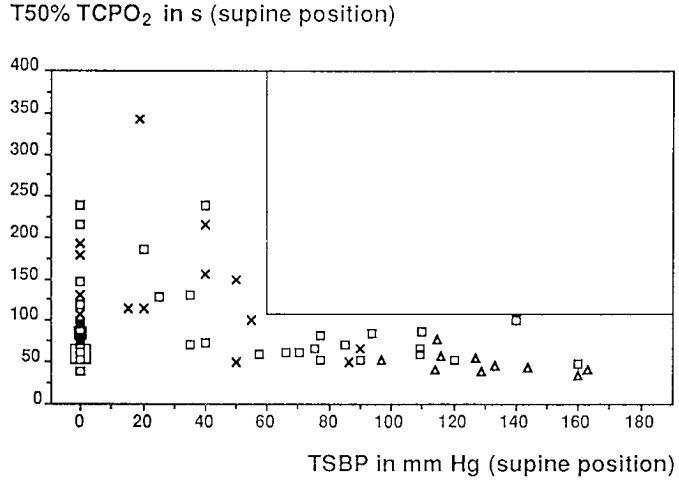


**Figure 5.2** Time needed to reach 50% of the RESTING $TCPO_2$ at the dorsum of the first metatarsophalangeal joint of the foot following the release of a transient arterial occlusion (T50% $TCPO_2$) in the supine position for toe systolic blood pressure (TSBP) in the supine position in healthy subjects ( Δ = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (□ = F2-group and × = F3/4- group. The size of the symbol corresponds to the number of data points that coincide at that location.

shows that skin oxygen supply at rest and hence total skin blood flow at rest is rather undisturbed in patients with a TSBP value higher than 60 mm Hg. This is in accordance with the finding of Krähenbühl and colleagues, who reported that a TSBP value of 50 mm Hg or higher was associated with a normal skin oxygen delivery at rest (9). In patients with TSBP values below 60 mmHg, the range of RESTING $TCPO_2$ values is even larger and varies from 0 to 75 mm Hg (Figure 5.1). This wide range of RESTING $TCPO_2$ values indicates that TSBP values below 60 mm Hg in patients with peripheral vascular disease do not predict skin oxygen delivery at rest and that oxygen supply at rest does not reflect the condition of the macrocirculation. The large number of patients (13 out of 16) suffering from moderate peripheral vascular disease (F2-group) with a TSBP value of 0 mm Hg and a rather normal skin oxygenation at rest (RESTING $TCPO_2 \geq 34$ mm Hg) can probably be explained by the fact that patients, in whom no pulsatility could be detected photoplethysmographically, were classified as having a TSBP of 0 mm Hg (chapter 3). In view of the RESTING $TCPO_2$ values, however, a considerable amount of blood has to reach the skin in these patients, which indicates that the mean arterial pressure is still substantial. The very low RESTING $TCPO_2$ values of 0 and 1 mm Hg in the presence of TSBP values ranging from 20 to 59 mm Hg in 5 patients with severe peripheral vascular disease (F3/4-group) is caused neither by overestimation of TSBP values (range of toe-to-brachial systolic blood pressure index: 12-32%) nor by an extremely low systemic level of skin oxygen delivery at rest (range of skin oxygen delivery at rest at the chest: 47-66 mm Hg). Since in other patients the same range of TSBP is associated with a quite normal skin oxygen delivery at rest, the extremely low amount of oxygen supplied to the skin at rest in these 5 patients can probably be explained by obstructions at the skin microcirculatory level. As shown in Figure 5.2, a TSBP value of 60 mm Hg is also a cut-off point as far as the response to the release of a transient arterial occlusion (T50% $TCPO_2$), which indirectly reflects the degree of arteriolar dilation at rest, is concerned.

In patients with a TSBP value higher than 60 mm Hg the degree of arteriolar dilation at rest appears to be equal to that in healthy subjects. In patients with a TSBP value below 60 mm Hg, however, T50% $TCPO_2$ indicates a normal or increased degree of arteriolar dilation at rest. In other words, in patients with peripheral vascular disease, who have a TSBP value higher than 60 mm Hg in the supine position, $tcpO_2$ monitoring in this position can be omitted, because a normal peripheral skin oxygen delivery at rest is to be expected. $TcpO_2$ monitoring in the supine position, however, is of importance in patients with TSBP values below 60 mm Hg, when one has to be informed of their skin oxygen supply at rest and hence their total skin blood flow at rest.

As shown in Figures 5.3-5.7, in the sitting position no relation exists between TSBP on the one hand, and nutritional skin blood flow at rest (NSBF), the degree of arteriolar dilation at rest (RBCVI, TTP), the diameter of the arteriolar limbs of

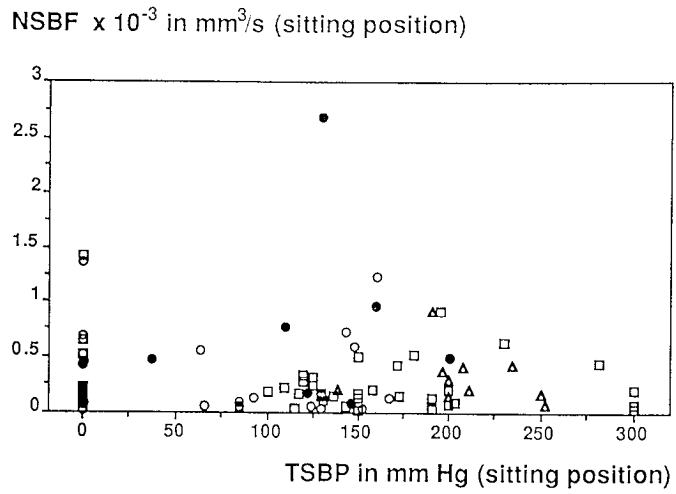


**Figure 5.3** Nutritional skin blood flow at rest per $mm^2$ of the investigated skin surface area (NSBF) in the sitting position for toe systolic blood pressure (TSBP) in the sitting position in healthy subjects (Δ = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (□ = F2-group, ○ = F3/4-INTACT-group and ● = F3/4-LESION-group). The size of the symbol corresponds to the number of data points that coincide at that location.

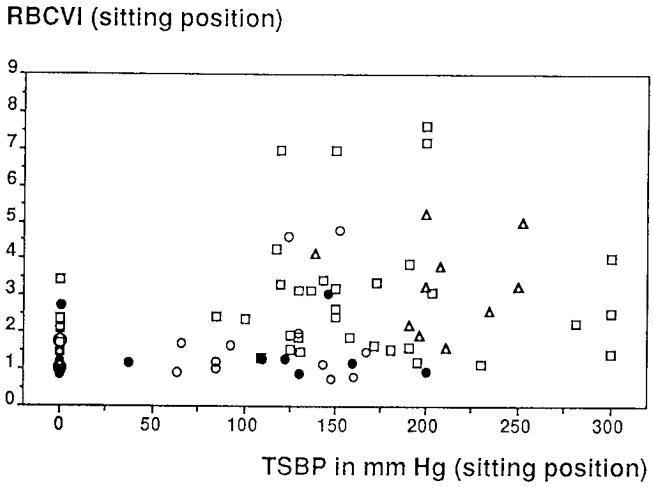


**Figure 5.4** Ratio of peak red blood cell velocity following the release of a transient arterial occlusion to red blood cell velocity at rest (RBCVI (red blood cell velocity index)) in the sitting position for toe systolic blood pressure (TSBP) in the sitting position in healthy subjects (Δ = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (□ = F2-group, ○ = F3/4-INTACT-group and ● = F3/4-LESION-group). The size of the symbol corresponds to the number of data points that coincide at that location.

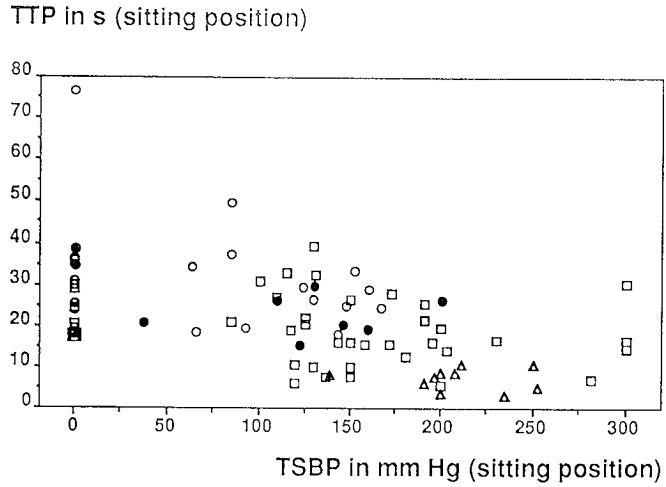


**Figure 5.5** Time-to-peak red blood cell velocity following the release of a transient arterial occlusion (TTP) in the sitting position for toe systolic blood pressure (TSBP) in the sitting position in healthy subjects (Δ = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (□ = F2-group, ○ = F3/4-INTACT-group and ● = F3/4-LESION-group). The size of the symbol corresponds to the number of data points that coincide at that location.

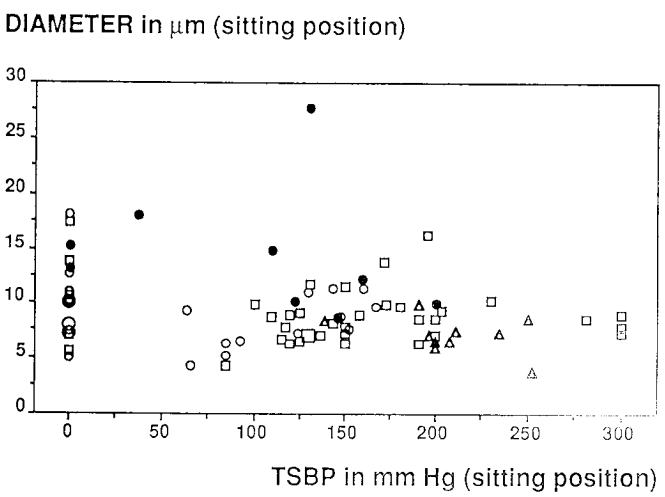


**Figure 5.6** Diameter of the arteriolar limbs of capillary loops (DIAM
for toe systolic blood pressure (TSBP) in the sitting position in hc
group) and in patients with various degrees of peripheral vas
Fontaine's classification (□ = F2-group, ○ = F3/4-INTACT-group ar
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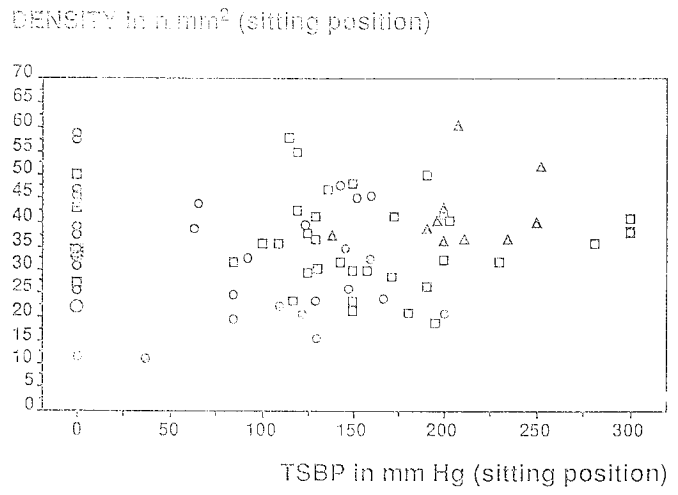


**Figure 5.7** Number of blood-filled capillaries per $mm^2$ of the investigated skin surface area (DENSITY) in the sitting position for toe systolic blood pressure (TSBP) in the sitting position in healthy subjects (Δ = control-group) and in patients with various degrees of peripheral vascular disease according to Fontaine's classification (□ = F2-group, ○ = F3/4-INTACT-group and ⊙ = F3/4-LESION-group). The size of the symbol corresponds to the number of data points that coincide at that location.

capillary loops (DIAMETER) and the number of blood-filled capillaries per $mm^2$ of the investigated skin surface area (DENSITY) on the other. Furthermore, in patients with peripheral vascular disease the significant difference in DIAMETER and DENSITY between intact skin parts (F2-group (□) and F3/4-INTACT-group (○)) and rims of ischaemic skin lesions (F3/4-LESION-group (⊙)) is not reflected in the TSBP nor is the significant difference in the degree of arteriolar dilation at rest (RBCVI) between patients with moderate (F2-group (□) and severe peripheral vascular disease (F3/4-INTACT-group (○) and F3/4-LESION-group (⊙).

These results indicate that in the sitting position no relation exists between the macrocirculation, as investigated with the use of TSBP measurements, and the nutritional skin microcirculation, as assessed by means of intravital skin capillary microscopy. In the sitting position macrocirculatory TSBP values do not provide information about the condition of the nutritional skin microcirculation. Irrespective of the outcome of TSBP measurements in the sitting position, intravital skin capillary microscopy should be performed to gain insight into the nutritional skin microcirculation.

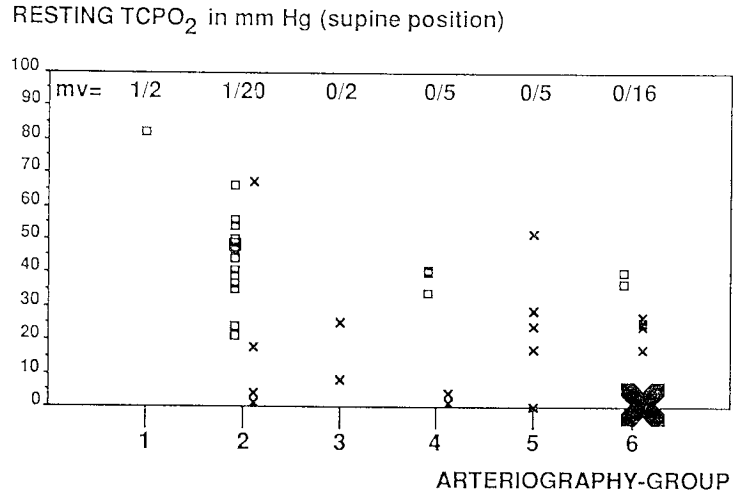


**Figure 5.8** Stable baseline $tcpO_2$ at rest at the dorsum of the first metatarsophalangeal joint of the foot (RESTING $TCPO_2$) in the supine position for the various ARTERIOGRAPHY-GROUPS, representing progressive arteriographic degrees of macrocirculatory obstruction; □ = F2-group, × = F3/4-group. The size of the symbol corresponds to the number of data points that coincide at that location; abscissa: 1 = ARTERIOGRAPHY-GROUP 1, 2 = ARTERIOGRAPHY-GROUP 2, etc; mv = missing value(s).

## 5.2 Relation between the skin microcirculation and arteriography

Scatter plots, displaying the relation between the degree of macrocirculatory obstruction, as visualized arteriographically, on the one hand, and total and nutritional skin blood flow at rest (RESTING $TCPO_2$ (supine) and NSBF (sitting), respectively) and the degree of arteriolar dilation at rest (T50% $TCPO_2$ (supine) and TTP (sitting), respectively) on the other, are shown in Figures 5.8-5.11. $TcpO_2$ parameters in the supine position and nutritional skin microcirculatory parameters in the sitting position are not significantly different in the various ARTERIOGRAPHY-GROUPS. This indicates that the arteriographic degree of macrocirulatory obstruction neither reflects the condition of the total skin microcirculation nor that of the nutritional skin microcirculation and vice versa.

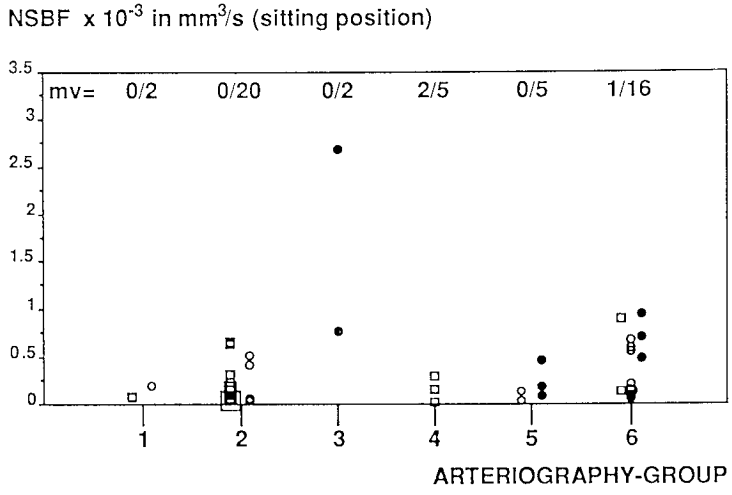


**Figure 5.9** Nutritional skin blood flow at rest per $mm^2$ of the investigated skin surface area (NSBF) in the sitting position for the various ARTERIOGRAPHY-GROUPS, representing progressive arteriographic degrees of macrocirculatory obstruction; □ = F2-group, ○ = F3/4-INTACT-group, ● = F3/4-LESION-group. The size of the symbol corresponds to the number of data points that coincide at that location; abscissa: 1 = ARTERIOGRAPHY-GROUP 1, 2 = ARTERIOGRAPHY-GROUP 2, etc; mv = missing value(s).

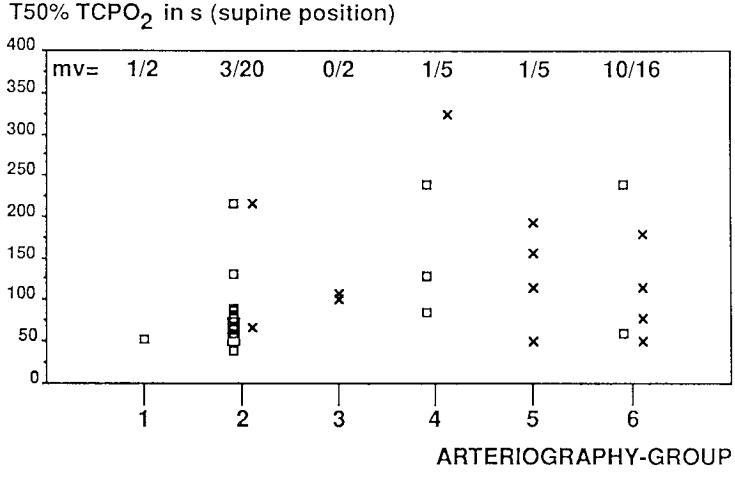


**Figure 5.10** Time needed to reach 50% of the RESTING $TCPO_2$ at the dorsum of the first metatarsophalangeal joint of the foot following the release of a transient arterial occlusion (T50% $TCPO_2$)in the supine position for the various ARTERIOGRAPHY-GROUPS, representing progressive arteriographic degrees of macrocirculatory obstruction; □ = F2-group, × = F3/4-group. The size of the symbol corresponds to the number of data points that coincide at that location; abscissa: 1 = ARTERIOGRAPHY-GROUP 1, 2 = ARTERIOGRAPHY-GROUP 2, etc; mv = missing value(s).

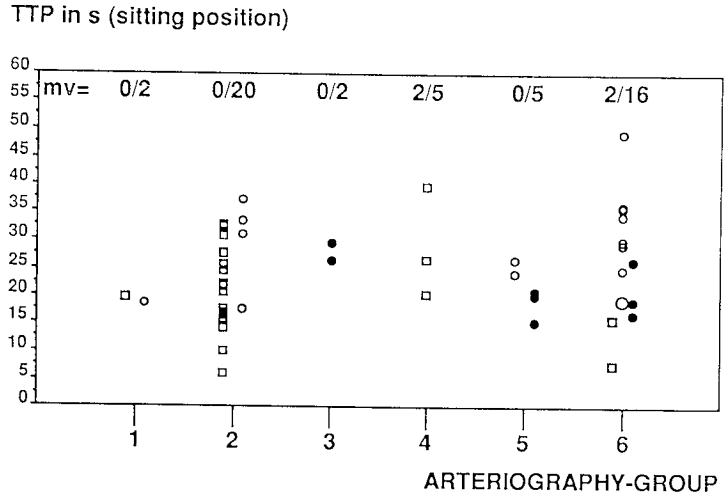


**Figure 5.11** Time-to-peak red blood cell velocity following the release of a transient arterial occlusion (TTP) in the sitting position for the various ARTERIOGRAPHY-GROUPS, representing progressive arteriographic degrees of macrocirculatory obstruction; □ = F2-group, ○ = F3/4-INTACT-group, ● = F3/4-LESION-group. The size of the symbol corresponds to the number of data points that coincide at that location; abscissa: 1 = ARTERIOGRAPHY-GROUP 1, 2 = ARTERIOGRAPHY-GROUP 2, etc; mv = missing value(s).

## 5.3 CONCLUSION

In patients with moderate and severe peripheral vascular disease (stages 2, 3 and 4 according to Fontaine) a TSBP higher than 60 mm Hg in the supine position is indicative of a relatively undisturbed total skin oxygenation at rest and hence a normal total skin blood flow at rest. TSBP values below 60 mm Hg, however, do not predict the level of total skin blood flow at rest nor does the degree of macrocirculatory obstruction, as visualized arteriographically. The necessity for $tcpO_2$ monitoring to be sure of an uncompromised total skin blood flow at rest in patients with moderate and severe peripheral vascular disease entirely depends on the outcome of TSBP measurements. Since TSBP, determined in the sitting position, and results of arteriography do not have any predictive value at all for the condition of the nutritional skin microcirculation, investigations with the use of intravital skin capillary microscopy are indispensable irrespective of the results of TSBP measurements and arteriography. In other words, intravital skin capillary microscopy is the only method presently available, that directly provides information about the condition of the nutritional skin microcirculation.

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# CHAPTER 6

# GENERAL DISCUSSION

In general, only the condition of the macrocirculation is investigated in patients suffering from atherosclerotic arterial obstructive disease of the lower limbs, who are routinely classified according to the stages of Fontaine (1). At present, techniques like Doppler ultrasound, plethysmography and arteriography are used to obtain information about the condition of the macrocirculation. Ischaemic skin lesions in patients with severe peripheral vascular disease are indicative of a disturbance at the level of the skin microcirculation in addition to a compromised macrocirculation. Consequently, macrocirculatory as well as skin microcirculatory investigations should be performed to evaluate the total vascular state of patients suffering from peripheral vascular disease. The skin microcirculation, which consists of a nutritional and a thermoregulatory part, is referred to as total skin microcirculation. The nutritional part of the skin microcirculation, which comprises the capillaries providing the nutrition of the skin, can directly be visualized and investigated with the use of intravital skin capillary microscopy. Information about the total skin microcirculation can be obtained indirectly by monitoring transcutaneous oxygen pressure ($tcpO_2$). Both skin microcirculatory techniques are non-invasive.

The aims of this thesis were 1) to investigate both the macrocirculation, and the total and nutritional skin microcirculation in patients with various degrees of lower limb ischaemia according to the classification of Fontaine by means of the aforementioned techniques, and 2) to obtain information about the relation, if any, between macrocirculatory data on the one hand and total and nutritional skin microcirculatory data on the other. An additional aim of this thesis was to gain more insight into the ways, in which the skin microcirculation can adjust to macrocirculatory impairments.

The present study shows that arteriolar dilation serves as a functional adjustment at the precapillary level trying to maintain adequate nutritional skin blood flow at rest and hence to prevent skin damage in case of deteriorating blood supply due to arterial vascular disease. If, however, arterial vascular disease is severe and arterioles have reached their maximum dilation, progression of the disease probably results in insufficient nutritional skin blood flow at rest and hence the

development of ischaemic skin lesions. In response to tissue damage, capillaries are able to increase their diameter in an attempt to lower peripheral resistance and hence improve nutritional skin blood flow at rest.

The results of haemodynamic macrocirculatory investigations in healthy subjects and patients suffering from peripheral vascular disease show that the condition of the lower limb macrocirculation is best reflected in the level of the toe systolic blood pressure (TSBP), as determined in the sitting position (chapter 3). Ankle systolic blood pressure (ASBP) and hence ankle-to-brachial systolic blood pressure index (ABI) appears to be artificially elevated, due to the presence of arterial wall rigidity (Mönckeberg's sclerosis), in a considerable number of patients with severe peripheral vascular disease (24%), all of whom except one suffer from diabetes mellitus. This finding questions the general applicability of ASBP and ABI measurements to assess the quality of the macrocirculation in patients with severe peripheral vascular disease and especially in those with diabetes mellitus (chapter 3). Because rigidity of digital arteries due to calcification is uncommon and hence artificial elevation of TSBP is unlikely, TSBP measurements, performed in the sitting position, are preferred to ABI measurements to assess the condition of the macrocirculation in general and especially in patients with severe peripheral vascular disease suffering from diabetes mellitus (chapter 3) (2). Arteriography only gives anatomic information about the localization and extent of macrocirculatory obstructions. It does not provide haemodynamic information, as indicated by the considerable overlap between the degrees of macrocirculatory obstruction, as visualized arteriographically, and the outcome of ASBP and TSBP, and ABI and TBI (toe-to-brachial systolic blood pressure index) measurements (chapter 3).

The present study indicates that an impaired macrocirculation, as investigated with the use of TSBP measurements, is not necessarily related to a compromised total skin perfusion, as assessed with the use of transcutaneous $pO_2$ ($tcpO_2$) monitoring with a heated $tcpO_2$ electrode (chapter 5). A TSBP value of 60 mm Hg in the supine position appears to be a cut-off point as far as total skin blood flow at rest, measured by $tcpO_2$ monitoring, is concerned (chapter 5). In patients suffering from peripheral vascular disease a TSBP higher than 60 mm Hg is indicative of a normal total skin blood flow at rest. Below a TSBP value of 60 mm Hg, however, TSBP has no predictive value anymore and $tcpO_2$ monitoring is necessary to assess the extent of total skin perfusion at rest.

The results of intravital skin capillary microscopy suggest that in the sitting position with the legs dependent nutritional skin blood flow at rest in the intact skin of the foot of patients with severe peripheral vascular disease does not differ significantly from that of healthy subjects and patients with less severe peripheral vascular disease, despite their severely impaired macrocirculatory blood supply (chapter 4). Under disturbed macrocirculatory circumstances nutritional skin blood flow at rest can be adequately maintained by arteriolar dilation, preventing the development of skin damage. The increase in red blood cell velocity following

the release of a transient arterial occlusion in patients with severe peripheral vascular disease appears to be extremely low (chapter 4). This indicates that their arterioles at rest are almost dilated at the maximum and hence their arteriolar reactivity at rest is diminished considerably. Furthermore, it confirms that vasoconstrictive mechanisms, which are normally elicited upon leg dependency, are seriously impaired, if not abolished, in patients with severe peripheral vascular disease (3-9). A decrease in arteriolar reactivity, however, appears to be reversible, as shown in a recent study of Jacobs and colleagues (10). In their study, the post-occlusive reactive hyperaemia response was improved after successful reconstructive vascular surgery, indicating arteriolar dilation at rest to be decreased. Since the present study indicates that at rest the arterioles in the intact skin of patients suffering from severe peripheral vascular disease are almost dilated at the maximum and no additional compensatory mechanism is available at the precapillary level anymore, a (further) reduction in nutritional skin blood flow at rest, caused by a progression of the disease and/or external skin compression, is likely to result in the development of ischaemic skin lesions.

In the sitting position the number of blood-filled capillaries and hence the surface area for exchange in the rims of ischaemic skin lesions appears to be significantly decreased, as compared to intact skin parts in patients with the same degree of peripheral vascular disease (chapter 4). The significant increase in the diameter of capillaries in the rims of ischaemic skin lesions probably compensates for the considerable decrease in the number of capillaries, as far as the surface area for exchange is concerned (chapter 4). This idea is supported by the slightly higher nutritional skin blood flow at rest in these rims, as compared to the nutritional skin blood flow at rest in the intact skin. An increase in nutritional skin blood flow at rest in the rims of ischaemic skin lesions by means of an adjustment at the capillary level, which is probably caused by vasodilating metabolites released from these damaged skin parts, might serve a twofold function: 1) prevention of further enlargement of the ischaemic skin lesion and 2) healing of the ischaemic skin lesion. The increase in capillary diameter in the rims of ischaemic skin lesions is in accordance with the finding of Peeze Binkhorst and colleagues in an animal study, showing an increase in capillary lumen adjacent to degenerated and leukocyte-invaded muscle fibers (11). They speculated that the increase in cross-sectional capillary luminal area represented a dimensional reserve to be utilized for increased capillary blood flow.

Nutritional skin blood flow at rest appears to be maintained in the sitting position. This may be beneficial to patients with severe peripheral vascular disease. Therefore, these patients are advised to assume a sitting position with the legs dependent during the day while intermittently activating their venous calf pump by means of ankle dorsiflexion and plantar flexion to lower venous pressure, to sleep with their feet slightly below heart level (anti-Trendelenburg's position), and not to wear pinching shoes.

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# CHAPTER 7

# SUMMARY

An inadequate blood supply to the lower limbs (ischaemia), due to peripheral arterial obstructive disease, often leads to pain during exercise (intermittent claudication) or even at rest. At present, only the macrocirculation, responsible for the transport of blood, is routinely investigated in patients suffering from peripheral vascular disease, although the occurrence of ischaemic skin lesions (ulcers and/or necrosis) indicates an impaired nutritional skin microcirculation. The nutritional skin microcirculation represents the level, at which nutrients and waste products are exchanged between blood and tissue.

As described in **chapter 1**, this thesis not only discusses the results of macrocirculatory and skin microcirculatory investigations in patients with various degrees of peripheral vascular disease, it also reports on the relation between the macrocirculation and the skin microcirculation and especially on the way, in which the skin microcirculation is able to adapt to changes in macrocirculatory blood supply. In this study, the macrocirculation was investigated with the use of Doppler ultrasound, plethysmography and arteriography. The nutritional part of the skin microcirculation, which consists of capillaries providing the nutrition of the skin, was examined with the use of intravital skin capillary microscopy. Transcutaneous oxygen pressure ($tcpO_2$) monitoring was applied to be informed of the total skin microcirculation, which comprises the abovementioned nutritional part and a thermoregulatory part, consisting of arteriovenous anastomoses, and arterial and venous plexuses.

In **chapter 2**, the subjects, who participated in this study, are presented. Thirty-nine patients with intermittent claudication (stage 2 according to Fontaine's classification = moderate peripheral vascular disease), 38 patients suffering from rest pain or ischaemic skin lesions with or without rest pain (stages 3 and 4 according to Fontaine's classification = severe peripheral vascular disease) and 10 healthy control subjects were examined in this study.

**Chapter 3** deals with the investigation of the lower limb macrocirculation. Following historical reviews and descriptions of the investigative methods, the results are presented and discussed. The toe systolic blood pressure, determined in the sitting position with the legs dependent, appeared to be the best variable to estimate the

degree of peripheral vascular disease, especially in diabetic patients. The arteriographic degree of macrocirculatory obstruction did not relate to the haemodynamic data, as obtained with the use of Doppler ultrasound and plethysmography.

**Chapter 4** reports on the investigation of the skin microcirculation. Historical reviews and descriptions of the techniques of $tcpO_2$ monitoring and intravital skin capillary microscopy precede data presentation and discussion. Total skin blood flow at rest, as reflected by $tcpO_2$ monitoring, was significantly impaired in patients with severe peripheral vascular disease. The time needed to reach 50% of the $tcpO_2$ value at rest following the release of a transient arterial occlusion appeared to provide the best separation between healthy subjects and patients with moderate peripheral vascular disease. Investigation of the nutritional skin microcirculation with the use of intravital skin capillary microscopy in the sitting position with the legs dependent showed that nutritional skin blood flow at rest in the intact skin of patients with severe peripheral vascular disease did not differ significantly from that of healthy subjects and patients with moderate peripheral vascular disease, despite their severely impaired macrocirculatory blood supply. Unlike in healthy subjects and patients with moderate peripheral vascular disease, however, only a slight increase in red blood cell velocity following the release of a transient arterial occlusion was observed in the intact skin of patients suffering from severe peripheral vascular disease. This indicates that at rest in the sitting position the arterioles in the intact skin of patients with severe peripheral vascular disease are considerably dilated to increase nutritional skin blood flow. Since this dilation is almost at the maximum and no other precapillary compensatory mechanism is available, a (further) decrease in nutritional skin blood flow at rest, for example, due to progression of peripheral vascular disease and/or local external skin compression, is likely to cause the development of ischaemic skin lesions. In the rims of ischaemic skin lesions a significant increase in the diameter of capillaries associated with a considerable decrease in the number of capillaries per surface area was seen. Since in these rims nutritional skin blood flow at rest appeared to be slightly higher, as compared to the nutritional skin blood flow at rest in the intact skin, the increase in diameter must probably be regarded as an ultimate capillary adjustment to increase nutritional skin blood flow at rest.

In **chapter 5**, the relation between the macrocirculation and skin microcirculation is discussed. A toe systolic blood pressure, determined in the supine position, higher than 60 mm Hg appeared to be indicative of a normal total skin blood flow at rest. Below 60 mm Hg, however, toe systolic blood pressure values did not predict the level of the total skin blood flow at rest. Toe systolic blood pressure values, determined in the sitting position with the legs dependent, nor the arteriographic degree of macrocirculatory obstruction had any predictive value for the level of the nutritional skin microcirculation, as determined in the sitting position.

In **chapter 6**, a general discussion about the most striking macrocirculatory and skin microcirculatory findings concludes this thesis.

# CHAPTER 8

# SAMENVATTING

Wanneer de benen onvoldoende van bloed worden voorzien (ischemie) door vernauwing van de bloed toevoerende vaten (perifeer arterieel obstructief vaatlijden) kan pijn onstaan tijdens het lopen (claudicatio intermittens) en zelfs in rust. In het algemeen worden alleen de grote bloed toevoerende vaten (macrocirculatie) routinematig onderzocht bij patiënten met perifeer arterieel vaatlijden, ofschoon het voorkomen van ischemische huidafwijkingen (ulcera en/of necrose) wijst op een gestoorde nutritieve huidmicrocirculatie. Op het niveau van de nutritieve huidmicrocirculatie vindt namelijk de uitwisseling van voedings- en afvalstoffen plaats.

Zoals beschreven in **hoofdstuk 1**, worden in dit proefschrift niet alleen de resultaten van het macrocirculatoire en huidmicrocirculatoire onderzoek bij patiënten met in ernst verschillend perifeer arterieel vaatlijden besproken, ook de relatie tussen de macrocirculatie en de huidmicrocirculatie en met name de manier waarop de huidmicrocirculatie zich kan aanpassen aan veranderingen in de macrocirculatoire bloedvoorziening komt aan de orde. De macrocirculatie werd onderzocht met behulp van Doppler ultrageluid, plethysmografie en arteriografie. Het nutritieve gedeelte van de huidmicrocirculatie, bestaande uit haarvaten (capillairen) die de huid van voedingsstoffen voorzien, werd bestudeerd met behulp van intravitaal capillairmicroscopie. Informatie over de totale huidmicrocirculatie, die naast het zojuist genoemde nutritieve gedeelte gevormd wordt door een temperatuurregulerend deel, bestaande uit arterioveneuze shunts, en arteriële en veneuze netwerken, werd verkregen door middel van het transcutaan meten van de zuurstofspanning ($tcpO_2$ meting).

In **hoofdstuk 2** worden de verschillende groepen van personen, die onderzocht werden, gepresenteerd. Negenendertig patiënten met claudicatio intermittens (stadium 2 volgens de classificatie van Fontaine = matig ernstig perifeer arterieel vaatlijden), 38 patiënten met rustpijn of ischemische huidafwijkingen met of zonder rustpijn (stadium 3 en 4 volgens Fontaine's classificatie = ernstig perifeer arterieel vaatlijden) en 10 gezonde, controle-personen participeerden in dit onderzoek.

**Hoofdstuk 3** beschrijft het macrocirculatoire onderzoek. Eerst worden historische overzichten en beschrijvingen van de onderzoeksmethoden gegeven, waarna de resultaten worden gepresenteerd en besproken. Het bleek, dat de systolische teenbloeddruk, gemeten in zittende houding met afhangend been, het beste de ernst van perifeer arterieel vaatlijden weerspiegelde en met name bij patiënten met diabetes mellitus. De arteriografisch beoordeelde ernst van de macrocirculatoire obstructie was niet gerelateerd aan hemodynamische gegevens, zoals verkregen met behulp van Doppler ultrageluid en plethysmografie.

In **hoofdstuk 4** komt het onderzoek van de huidmicrocirculatie aan de orde. Historische overzichten en beschrijvingen van de gebruikte technieken ($tcpO_2$ meting en intravitaal capillairmicroscopie) gaan vooraf aan de presentatie en bespreking van de data. De totale bloeddoorstroming van de huid in rust bleek beduidend lager te zijn bij patiënten met ernstig perifeer arterieel vaatlijden. De tijd, die nodig was om weer de helft van de oorspronkelijke $tcpO_2$ waarde in rust te bereiken na het opheffen van een tijdelijke volledige arteriële afsluiting, bleek het beste onderscheid te maken tussen gezonde controle-personen en patiënten met matig ernstig perifeer arterieel vaatlijden. Onderzoek van het nutritieve gedeelte van de huidmicrocirculatie in zittende houding met afhangend been toonde geen verschil aan tussen de doorbloeding van de intacte huid van gezonde controle-personen, patiënten met matig ernstig en patiënten met ernstig perifeer arterieel vaatlijden. De slechts geringe toename van de snelheid van de rode bloedlichaampjes (erythrocyten) in de intacte huid na het opheffen van een tijdelijke, volledige arteriële afsluiting bij patiënten met ernstig perifeer arterieel vaatlijden wijst erop, dat de arteriolen (voorlopers van de capillairen, precapillaire bloedvaten) in rust reeds vrijwel volledig verwijd zijn en dat in rust de huidmicrocirculatie, althans op precapillair niveau, bijna al z'n reserves heeft aangesproken. Het is zeer waarschijnlijk, dat bij afwezigheid van een ander precapillair aanpassingsmechanisme (verdere) afname van de nutritieve huiddoorbloeding, bijvoorbeeld door verergering van het vaatlijden of door locale druk van buitenaf, het ontstaan van ischemische huidafwijkingen veroorzaakt. In de rand van zulke ischemische huidafwijkingen werd enerzijds een afname van het aantal capillairen per oppervlak gezien, anderzijds een toename van de diameter van de nog resterende capillairen. Verder bleek in rust de nutritieve bloeddoorstroming in deze randen iets hoger te zijn in vergelijking met de intacte huid. Dit fenomeen wijst erop, dat een toename van de capillaire diameter hoogstwaarschijnlijk een laatste mogelijkheid is om de nutritieve huiddoorstroming te verbeteren.

In **hoofdstuk 5** wordt de relatie besproken tussen de macrocirculatie en de huidmicrocirculatie. Een systolische teenbloeddruk, gemeten in liggende houding, hoger dan 60 mm Hg, ging gepaard met een normale totale huiddoorbloeding. Echter systolische teenbloeddrukken lager dan 60 mm Hg waren niet voorspellend voor wat de totale huiddoorstroming betrof. De nutritieve huiddoor-

stroming bleek niet te zijn gerelateerd aan de systolische teenbloeddruk, gemeten in zittende houding met afhangend been, noch aan de arteriografisch beoordeelde ernst van de macrocirculatoire obstructie.

In het afsluitende **hoofdstuk 6** wordt ingegaan op de meest opvallende bevindingen.

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# CURRICULUM VITAE

Roeland C.Y. Beckers werd geboren op 11 februari 1959 te Maastricht. In 1978 behaalde hij het diploma Gymnasium ß aan het Henric van Veldeke College te Maastricht. Hij studeerde Duitse Taal- en Letterkunde aan de Katholieke Universiteit Nijmegen in de periode 1978-1979. Vanaf november 1979 tot september 1980 was hij leerling radiologisch laborant in het St.Gregorius de Grote Ziekenhuis te Brunssum. In 1980 begon hij met de studie Geneeskunde aan de Rijksuniversiteit Limburg te Maastricht, waar in 1986 het artsdiploma behaald werd. Vanaf oktober 1986 tot november 1989 verrichtte hij als research-assistent bij de afdeling Chirurgie van het Academisch Ziekenhuis Maastricht (hoofd: Prof.dr. G. Kootstra) het in dit proefschrift beschreven onderzoek. In 1990 werkte hij gedurende een aantal maanden als arts-assistent Chirurgie in het St. Jozefziekenhuis te Kerkrade. Vanaf februari 1991 is hij werkzaam als arts-assistent Dermatologie in het Academisch Ziekenhuis Leiden (hoofd: Prof.Dr. B.J. Vermeer), waar hij sinds 1 oktober 1991 in opleiding is tot dermatoloog.